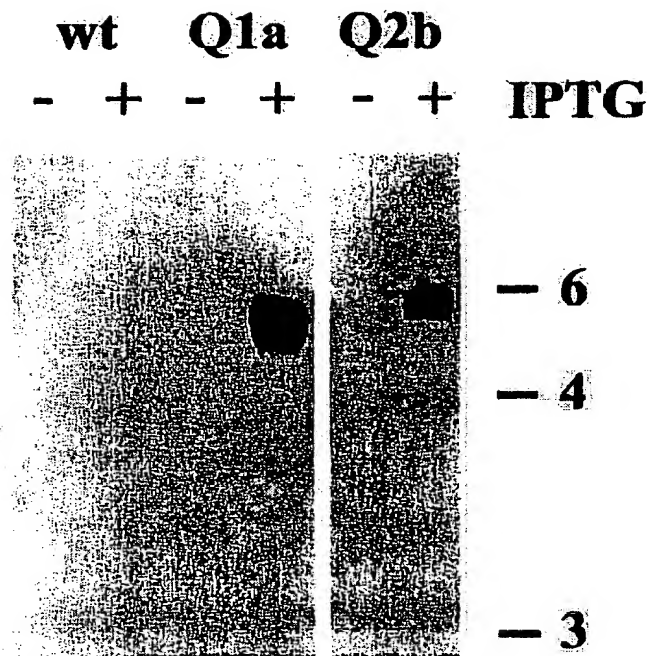
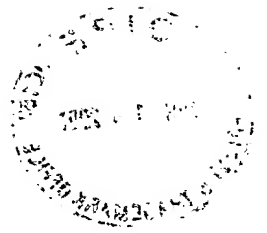


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**HCV E2 proteins detected
with mMAb E2G**

FIG. 1

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Sequences amplified from central region of HCV E2 vaccinia virus clones

>hcv-1a3, (Q1a)

```
CTCAACTGGATTACCCAAAGTGTGGGAGCGCCCCCTGTGTCATGGAGGGGGGG
CAACAACACCTT      GCGCTGCCCCACTGATTGTTCCGCAAGCATCCGGAAGCCAC
GTACTCTCGGTGCGGCTCCGGTCCCTGGATTACGCCCAAGGTGCCCTGGTC
```

>hcv-1b8, (Q1b)

```
TGGCACAGGGTTACCCAAAGACGTGTGGGGCCCCCCCATGTACATCGGGGGGTCGG
CAATAACACCTT      GACTTGGCCCCACGGACTGTTCCGGAAGCACCCCGAGGCCAC
TTACACCAAAATGTGTTCCGGGGCCTTGGCTGACACCTAGGTGCATAGTt
```

>hcv-2a-25, (Q2a)

```
CTCCACTGT TTCACCAAAACTTGGGGCGGCACCACCCTGCGGCATCAGAGCTGACTT
TAATGCCAGCACggaacctgctgtgccccCAGGACTGTTCAAGGAAGCATCCTGAAGCCAC
TTACATCAAATGTGGCTCTGGGCCctgtgacgccaagtgcctgata
```

>HCV-2B-1, (Q2b)

```
TGGGACTGGGTTCACTAAGACATGGGGTGCACCACCCTTGCCGCAATTAGGAGGGACTG
CAACGGAAACCTcgacctATTGTGCCCCACAGACTGTTTCAGAAAGCACCCAGATACTAC
CTACCTTAAGTGTGAGCGGGGCTTGTTGACCCCCCAAAATGCATGGTa
```

FIG. 2



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Name	Sequences
HCV-1a	CTCAACTGGA TTCACCAAG TGTGGGAGC GCCTCCTTGT GTATCGGAG GGGCGGCAA
HCV-Q1a-FRGT...GA C.....G C..C..G... AA.....G... ..TC..T..
HCV-1b	TAGT.....GT...GA C.....G C..C..G... AA.....G... ..TC..T..
HCV-Q1b-FR	TGGC..A..GGA C...T..G.. C..C..A... AA.....G... ..TC.....
HCV-2a	...C.....C .A.....GA CT.....C.. A..A..C..C CG...TA... CT.ACTT...
HCV-Q2a-FR	...C.....-TA CT.....C.. A..A..C..C CG...A... CT.ACTTT...
HCV-2b	.GGG.....GT..GA CA.....T.. A..A.....C CG...TA.GA AA.ACTA...
HCV-Q2b-FR	TGGG.....GT..GA CA.....T.. A..A.....C CG...TA.GA ...ACT....
HCV-1a	CAACACC--- ---CTGCACT GCCCCTGA TTGCTTCCGC AAGCATCCGG ACGCCACATA
HCV-Q1a-FRT...G... ..T.....G... ..T.....G... ..T.....G...
HCV-1b	.CG.....T...AT... ..G...C.....G... ..C..C..G..T..T...
HCV-Q1b-FR	T.....T...ACT... ..G...C..T.....G... ..C..C..G...T...
HCV-2a	TGC..G.ATG GACT..TTG.G..C..T..TA.G... ..TA....C...
HCV-Q2a-FR	TGC..G.ACG GAC...TG.G..C..T..A.G... ..T...A....T...
HCV-2b	..G...TATC GATT.ATTG.A..C..T..TA.G... ..C..A..T..T..C...
HCV-Q2b-FR	.GGA...CTC GAC.ATTG.A..C..T..A.A... ..C..A..TA.T..C...

FIG. 3A

Name

Sequences

HCV-1a	CTCTCGGTGC	GGCTCCGGTC	CCTGATCAC	ACCCAGGTGC	CTGGTC
HCV-Q1a-FRT..	G.....
HCV-1b	.A.AAAA..TG..G.T.G..	...T.....	..A..A
HCV-Q1b-FR	.A.CAAA..T	.T..G..G.	.T..C.G..	..T.....	A.A..T
HCV-2a	.ATCAAA..TT..G.C....	G..A.....	...A..
HCV-Q2a-FR	.ATCAAA..TT..G.	..CT..-G..	G..A.A....	...A.A
HCV-2b	TCT.AA...T	..AG.A..G.	.T..T.A..	T.....	...A
HCV-Q2b-FR	.CT.AA...T	..AG.G..G.	.T..T.G..	C...AA...	A...A

One most parsimonious tree found:

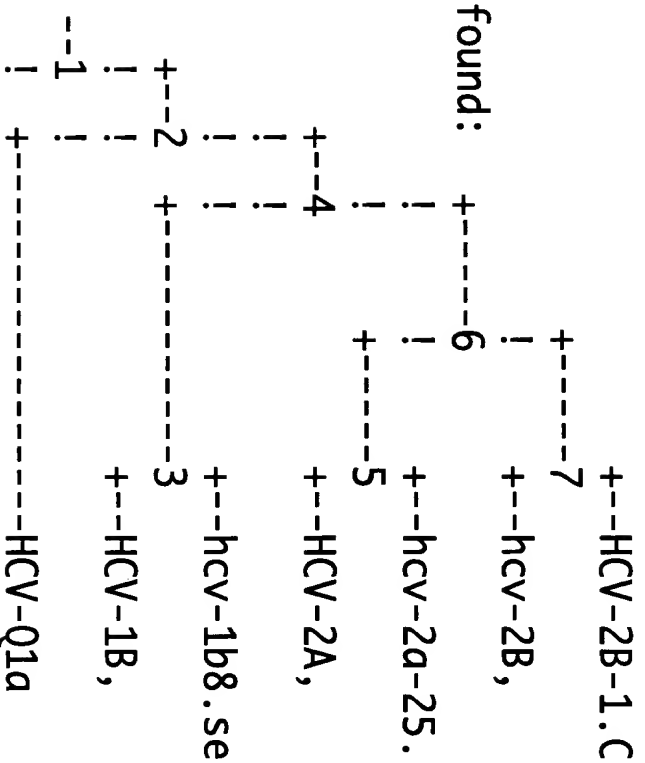


FIG. 3B

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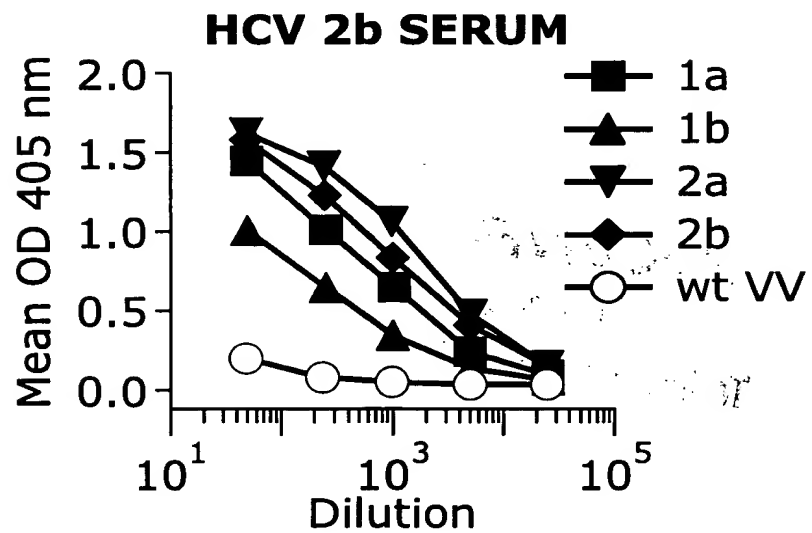
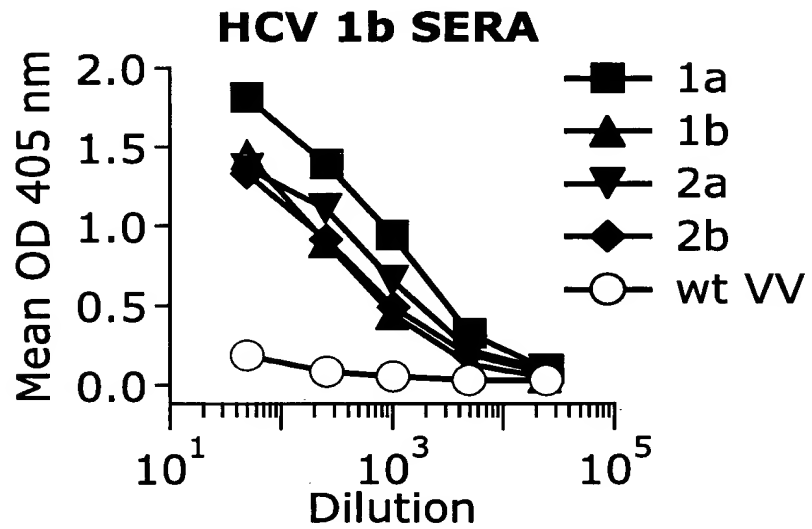
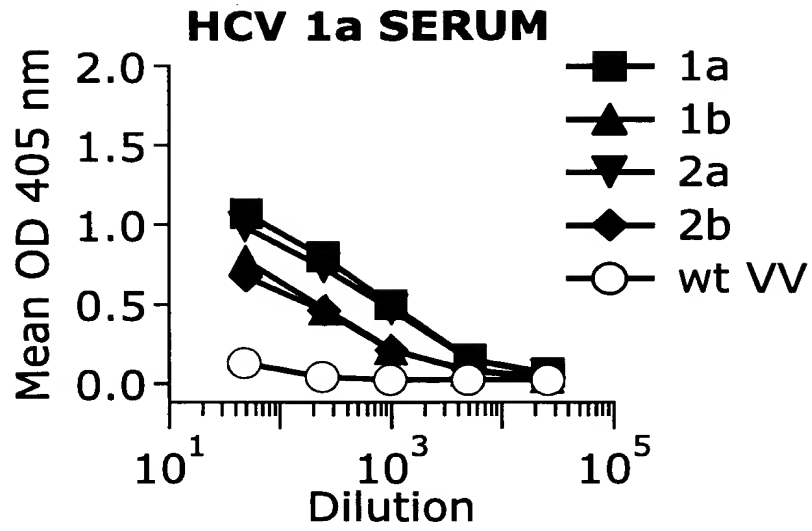


FIG. 4A

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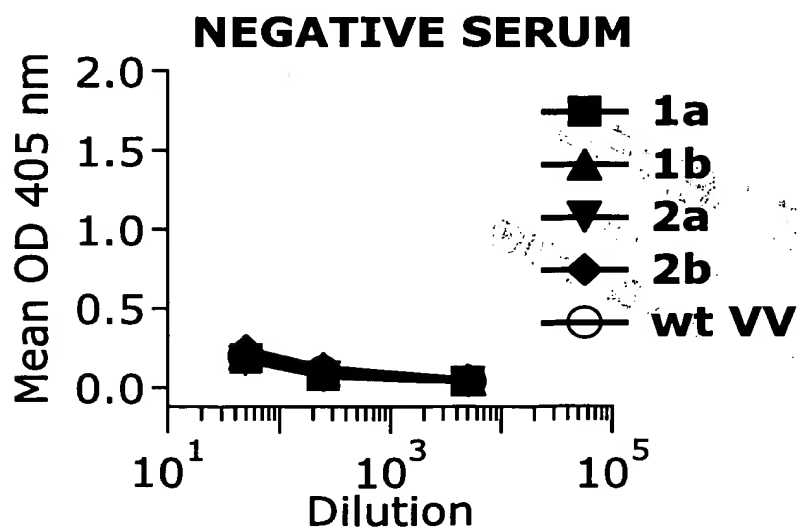
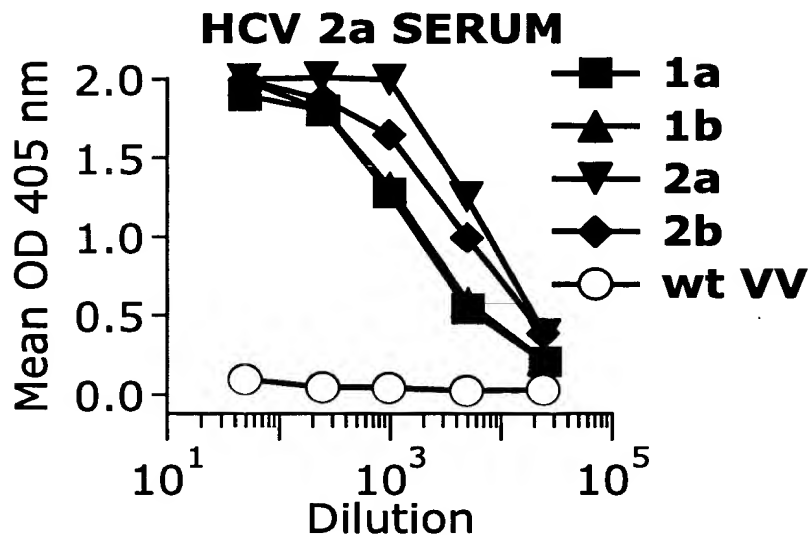
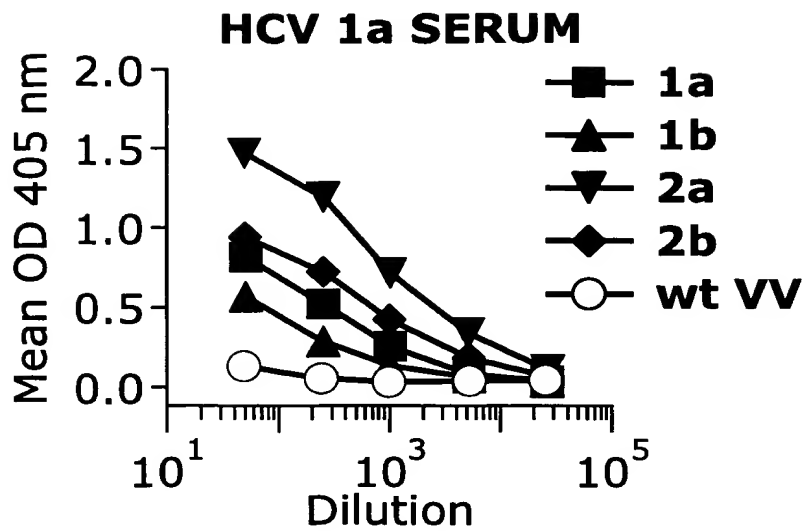


FIG. 4B

Reactivity obtained with 12 HCV 2b Sera

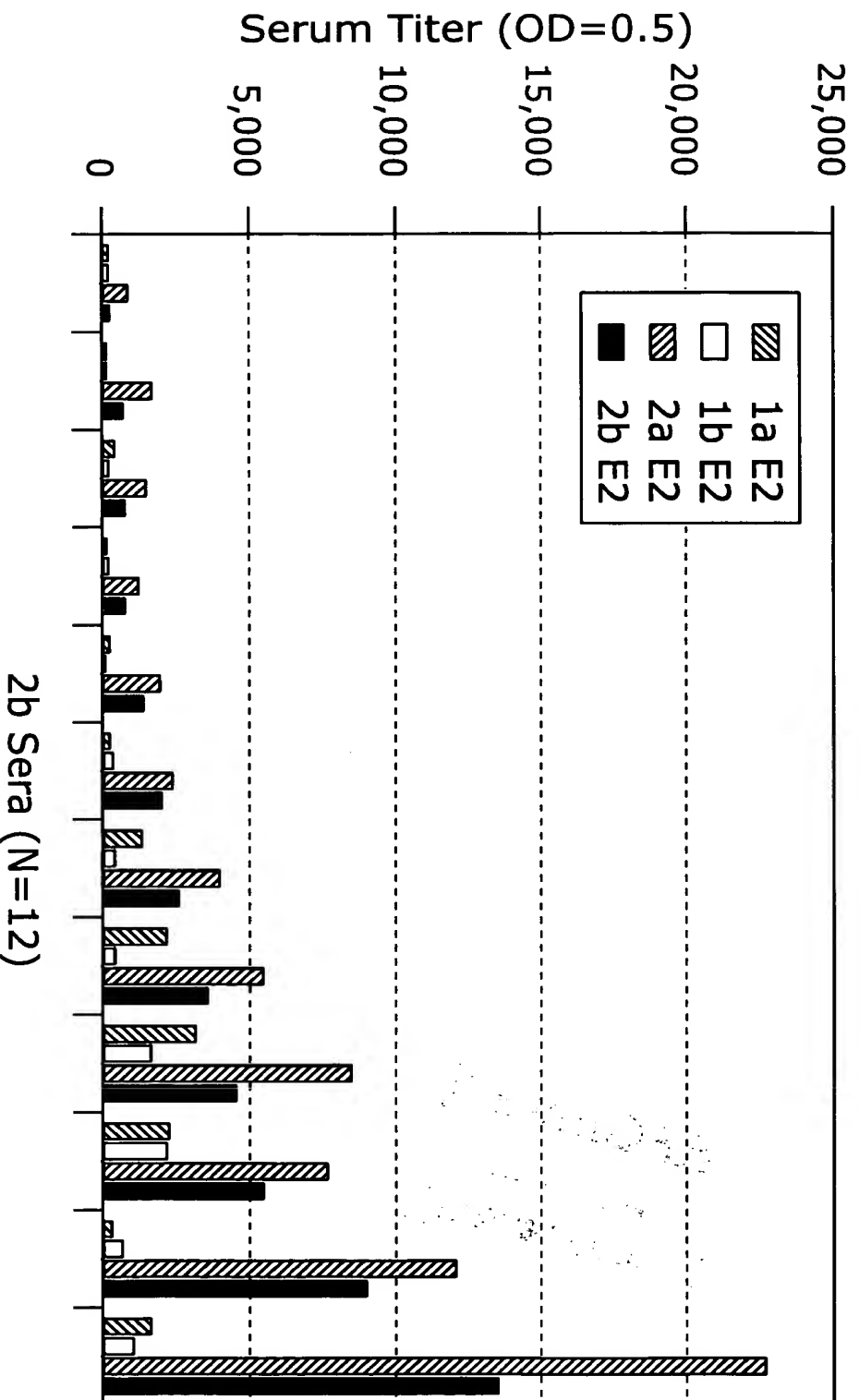
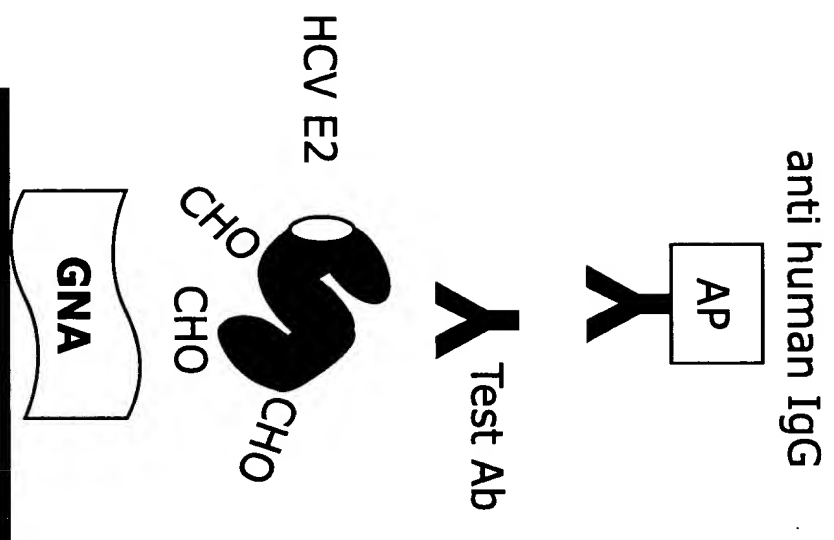


FIG. 5

Binding Analysis of HCV HMABs



- Coat plates with GNA
- Add E2 containing protein extracts
- Add MAb
- Detect bound Ab with anti Human-AP

FIG. 6



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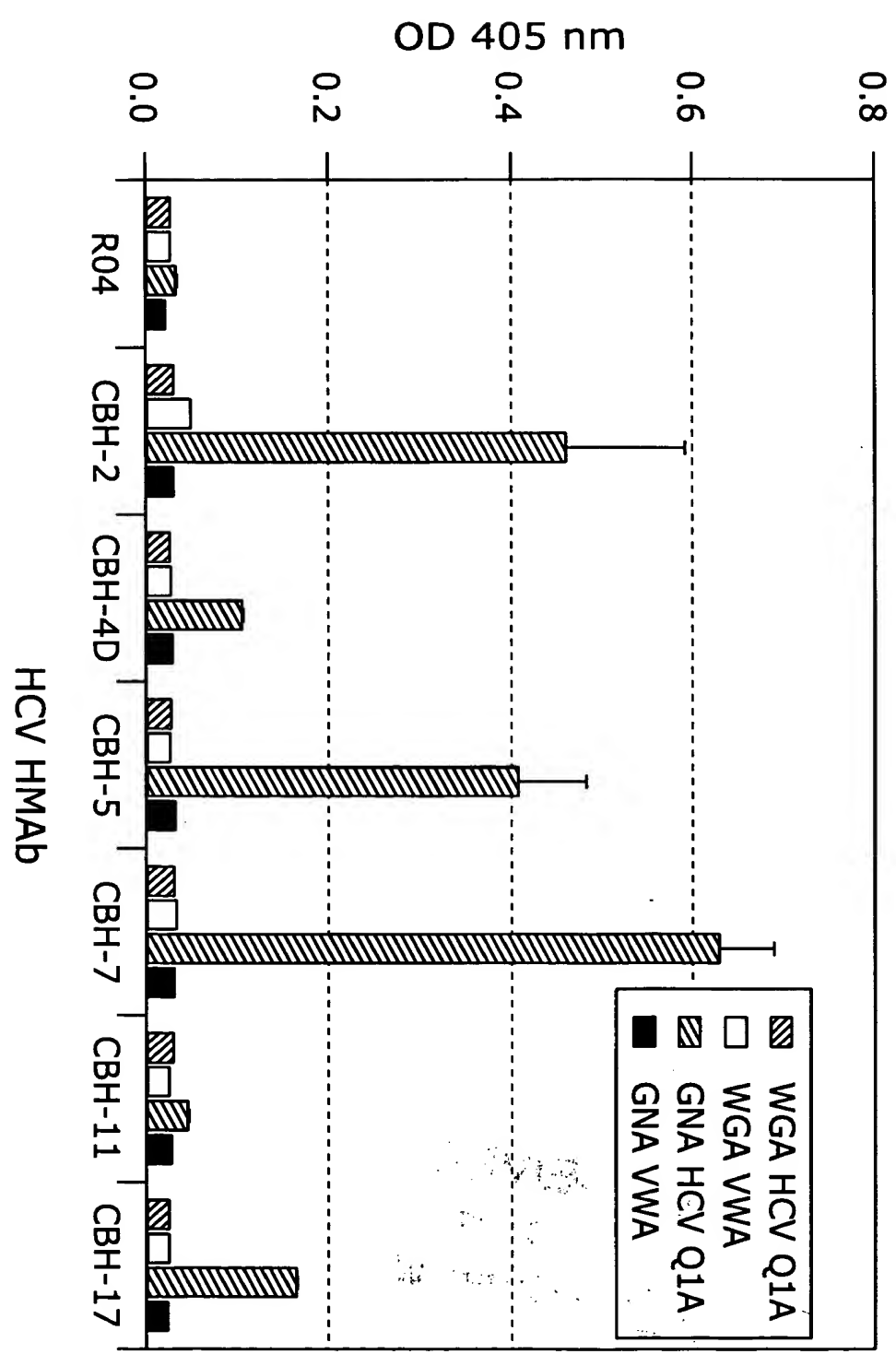


FIG. 7



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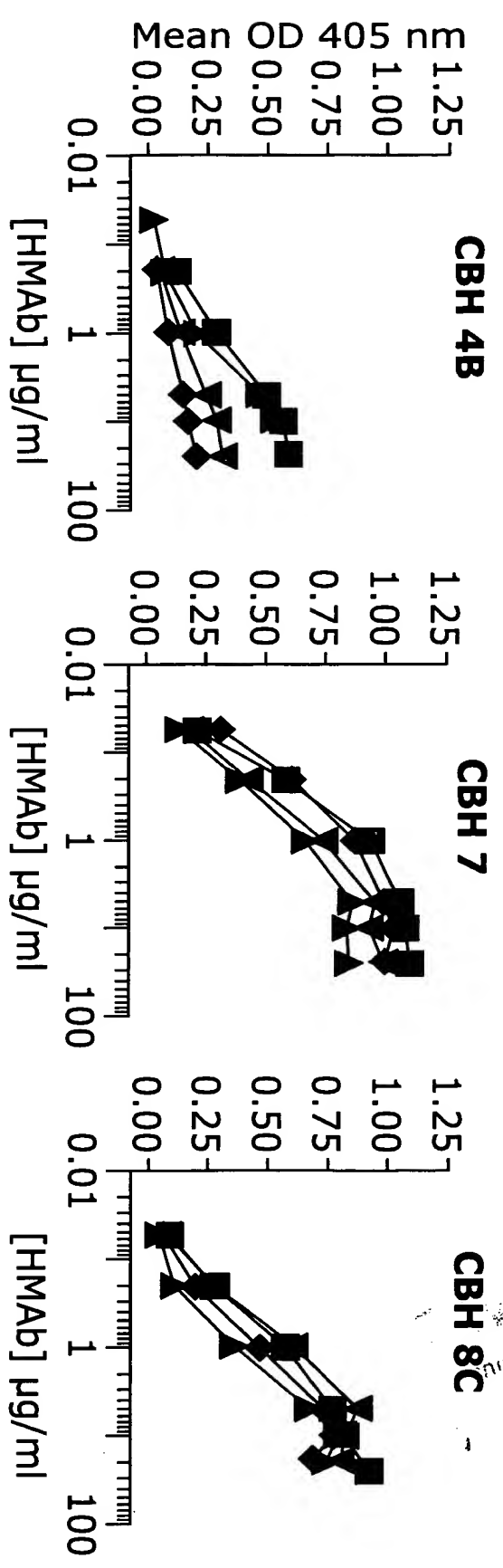
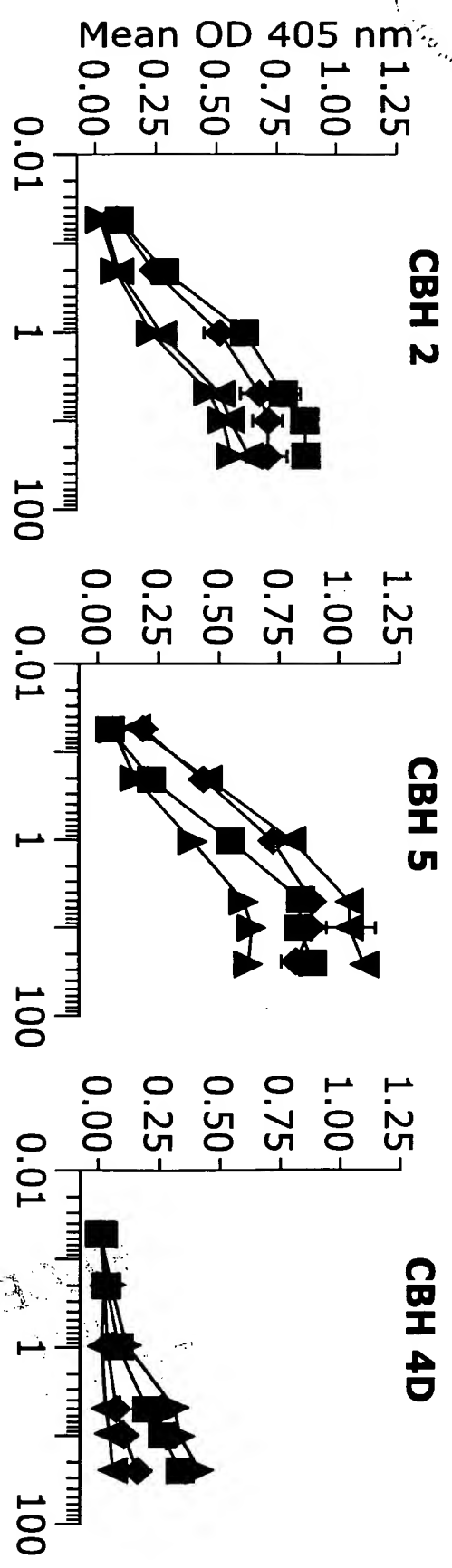


FIG. 8A



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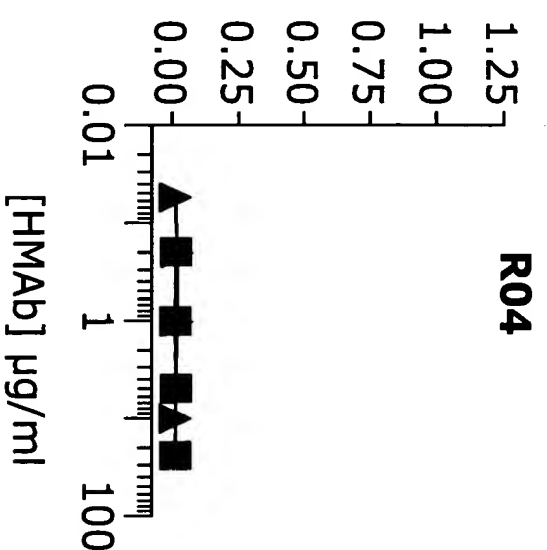
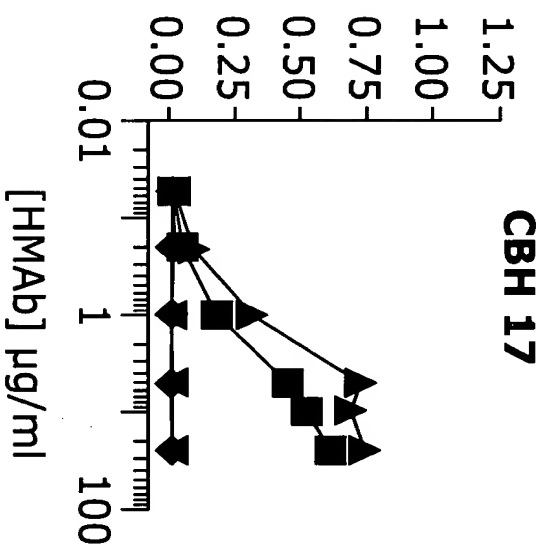
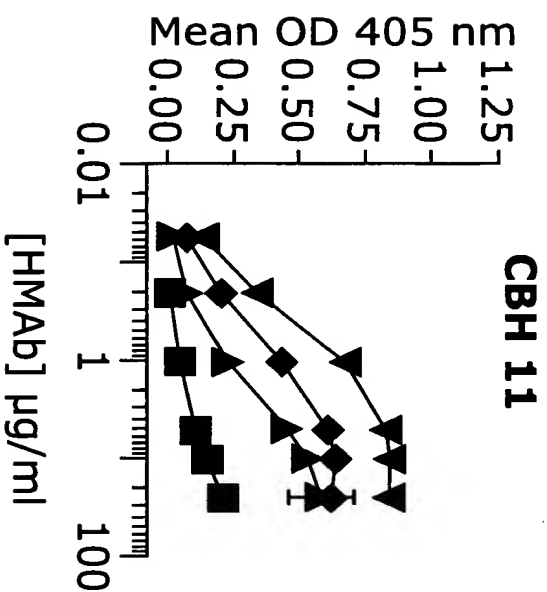
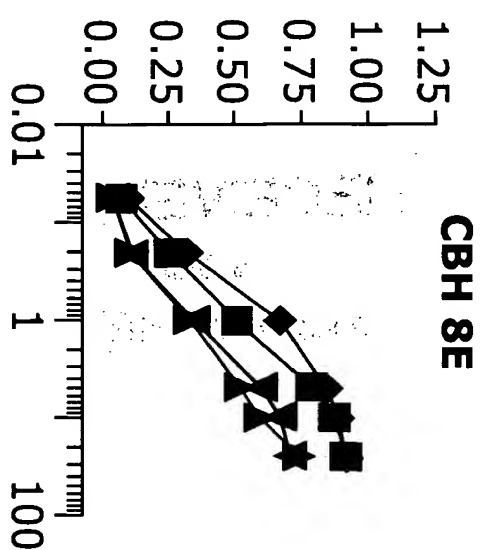
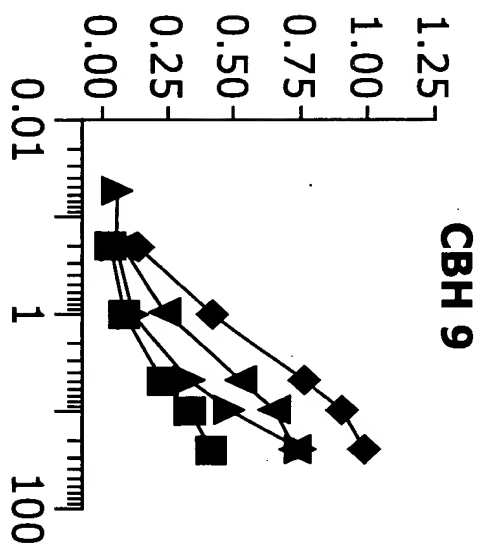
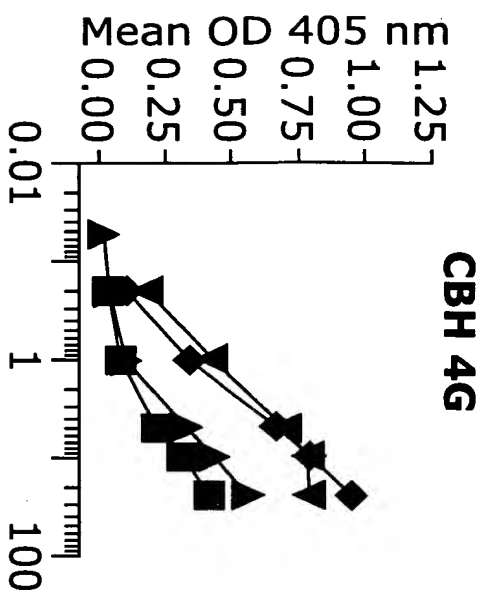


FIG. 8B

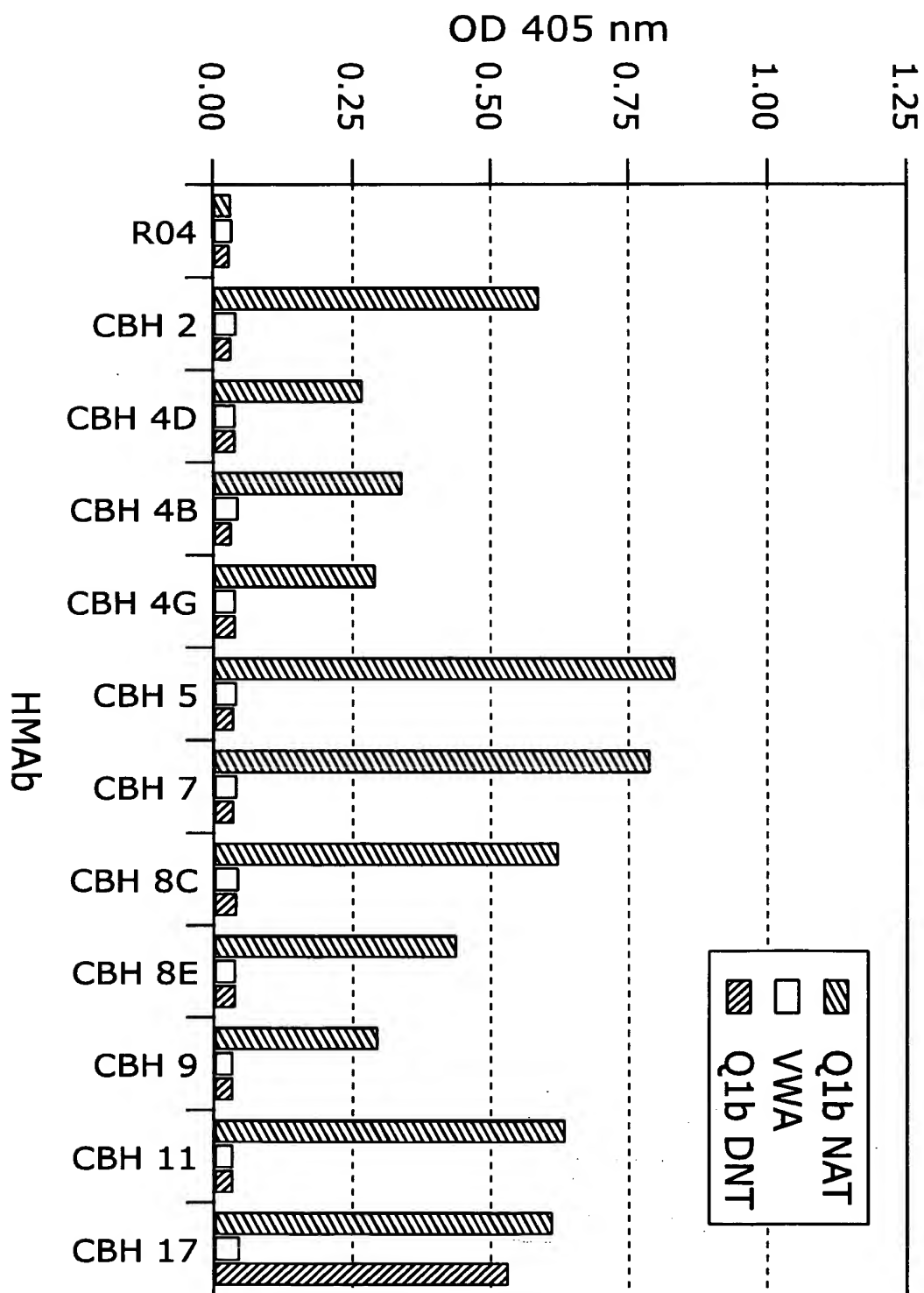
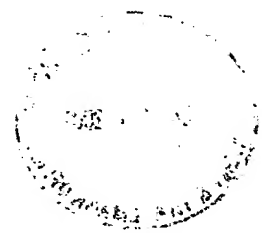
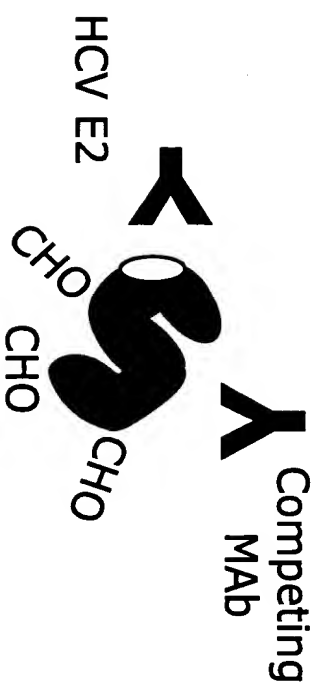
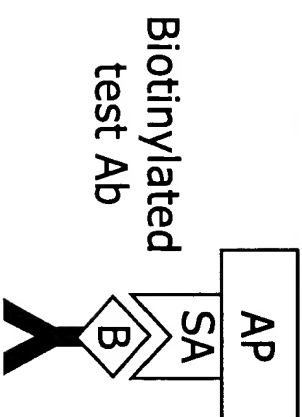


FIG. 9



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Competition Analysis



- Coat plates with GNA lectin
- Add E2 containing protein extracts

- Add competing MAb
- Add biotinylated Test HMab
- Detect bound Ab with Strep-AP

FIG. 10



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CBH 5 Competition Analysis

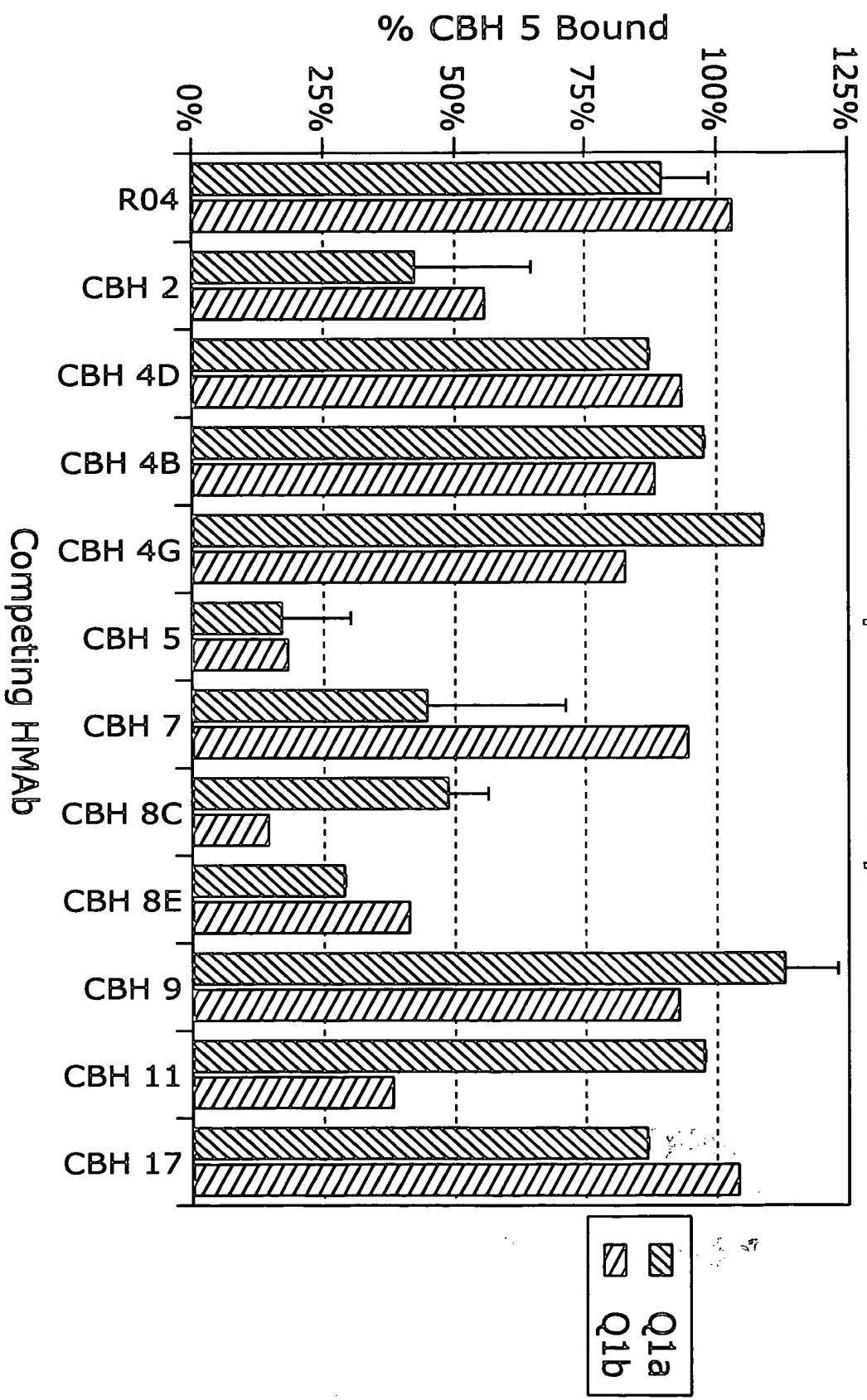


FIG. 11



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CBH 2 Competition Analysis

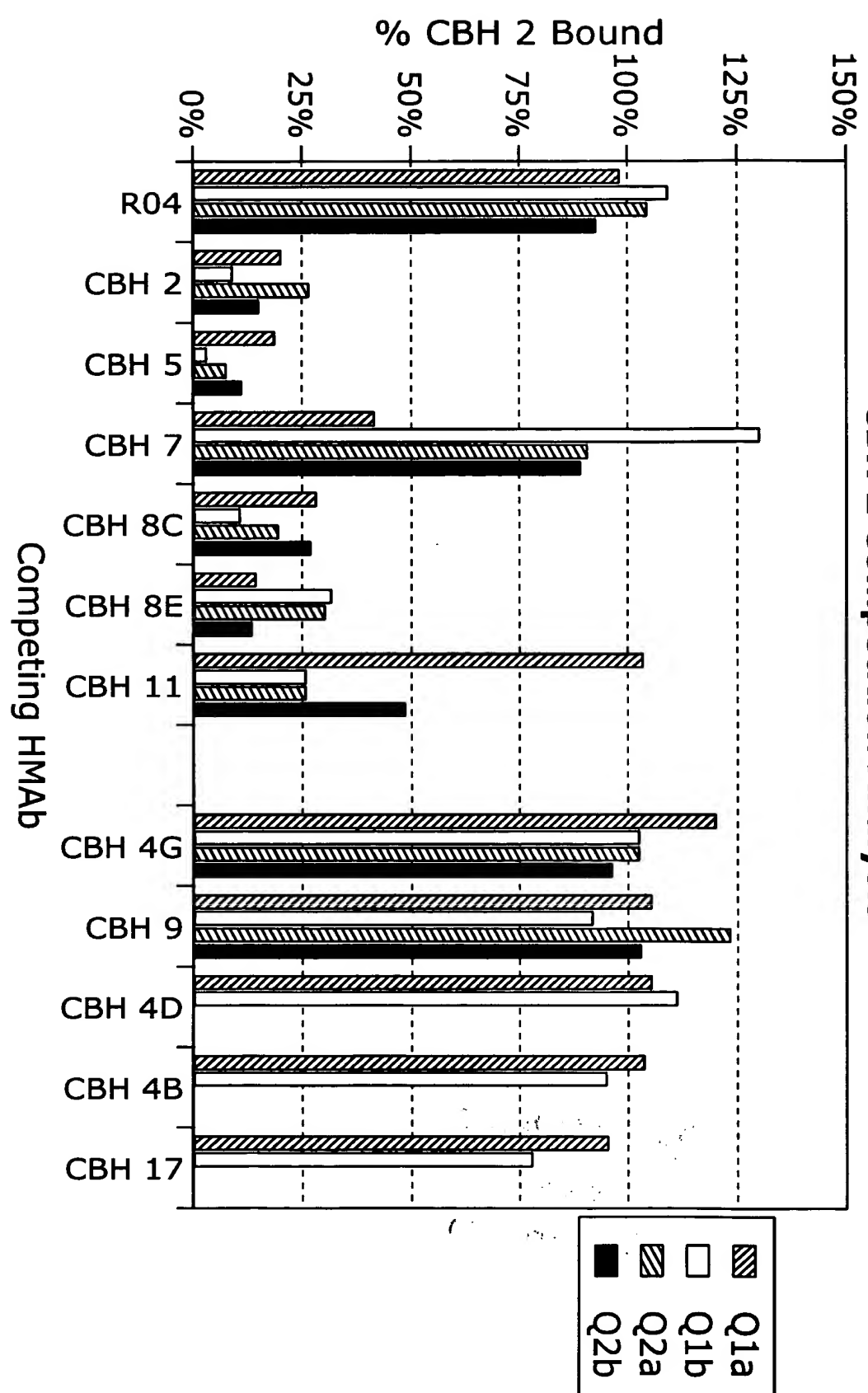


FIG. 12



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CBH 7 Competition Analysis

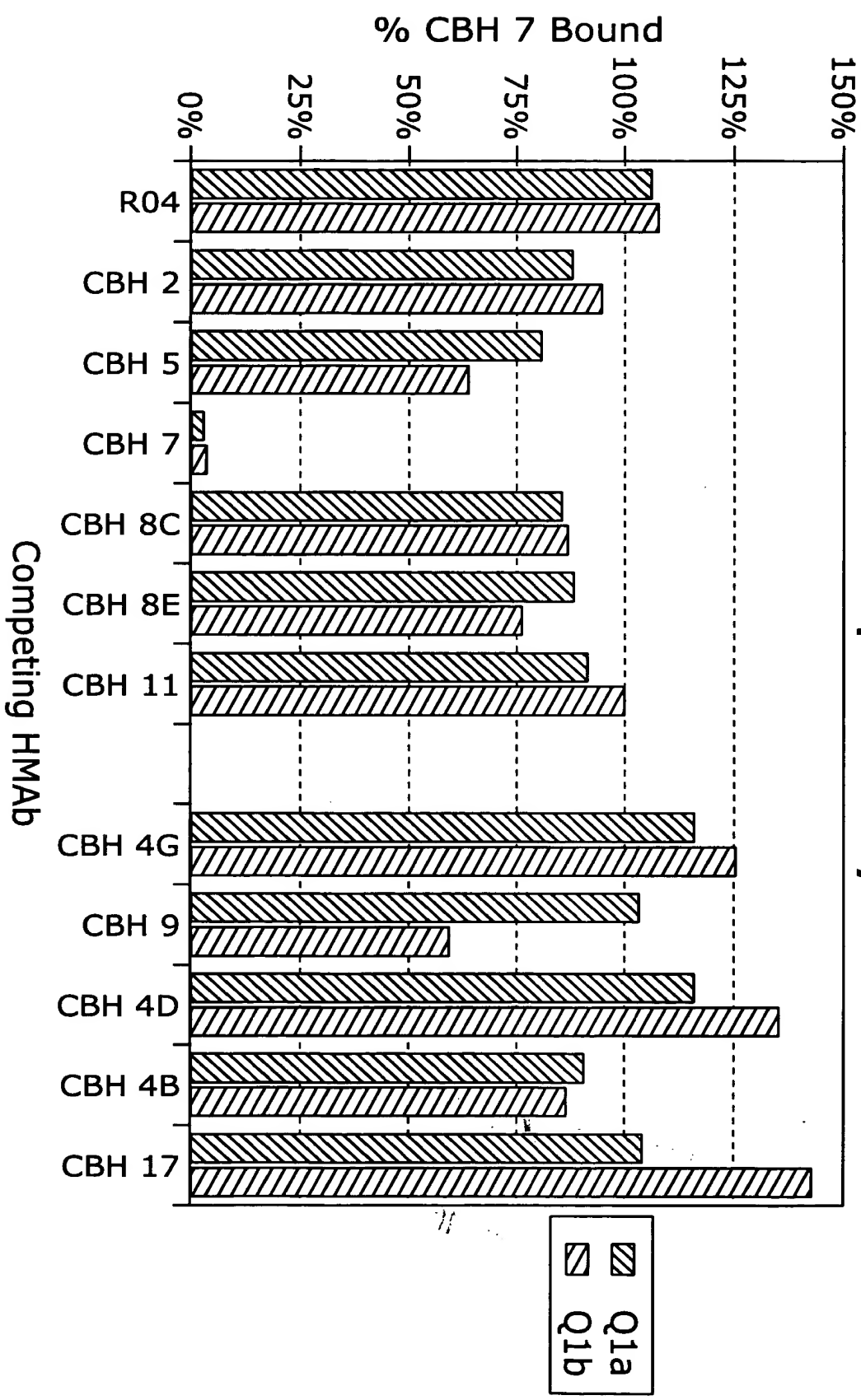
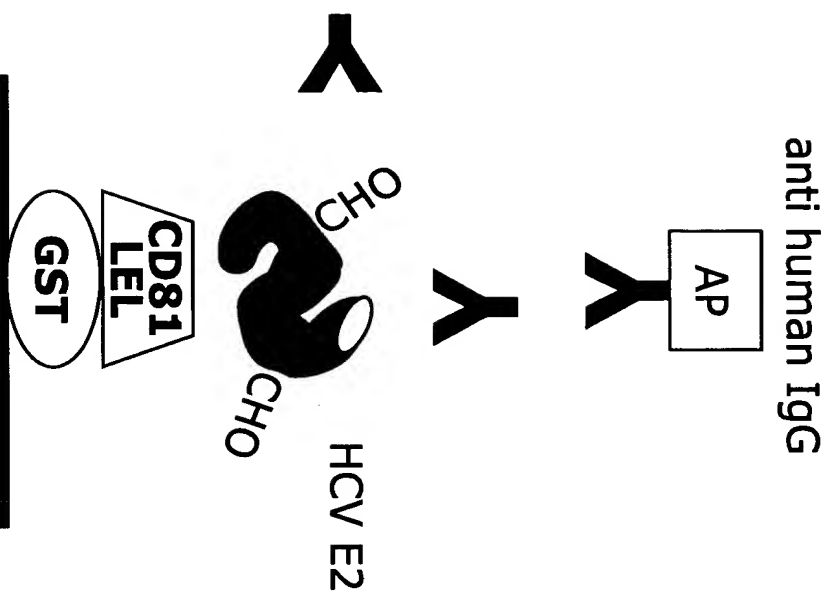


FIG. 13

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Binding to CD81-E2 complex



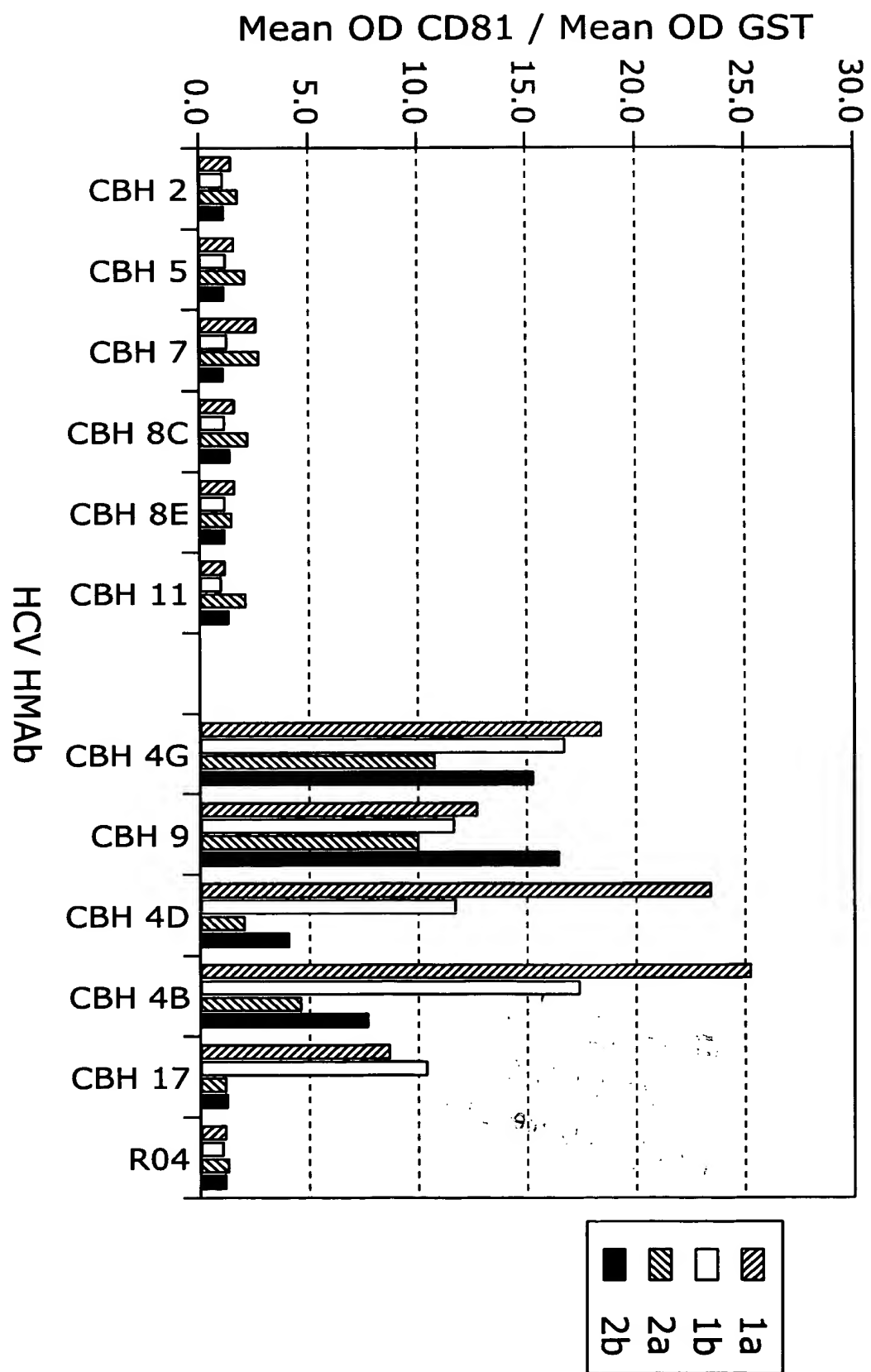
- Coat plates with GST-CD81-LEL or GST
- Add E2 containing protein extracts
- Add MAb
- Detect bound Ab with anti Human-AP

FIG. 14



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FIG. 15



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Inhibition of HCV virion binding to CD81

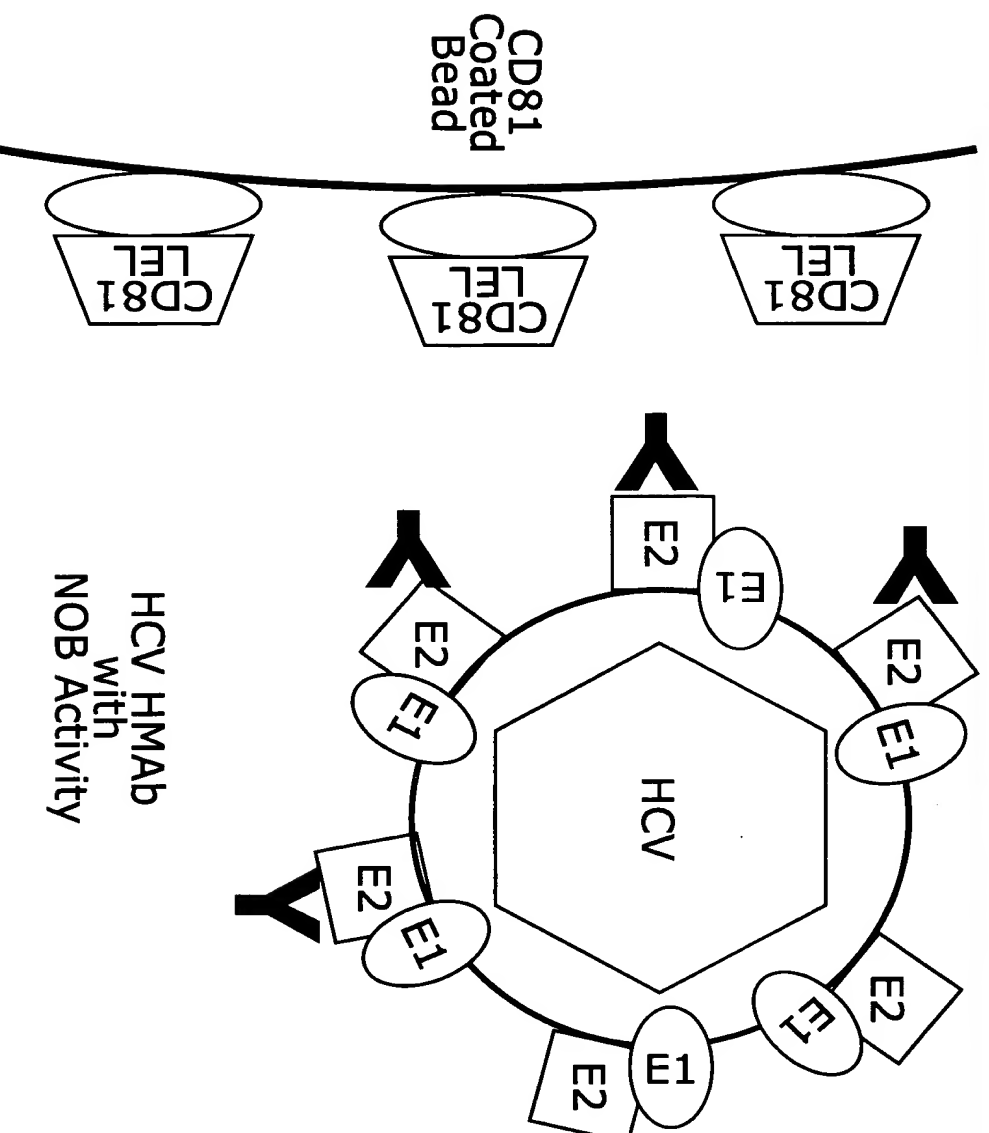


FIG. 16

- Coat beads with CD81
- Combine serum with known amount of HCV virions with test HCV HMAb
- Incubate with beads
- Pellet & wash beads
- Detect bound HCV virions by quantitative PCV (Taqman)



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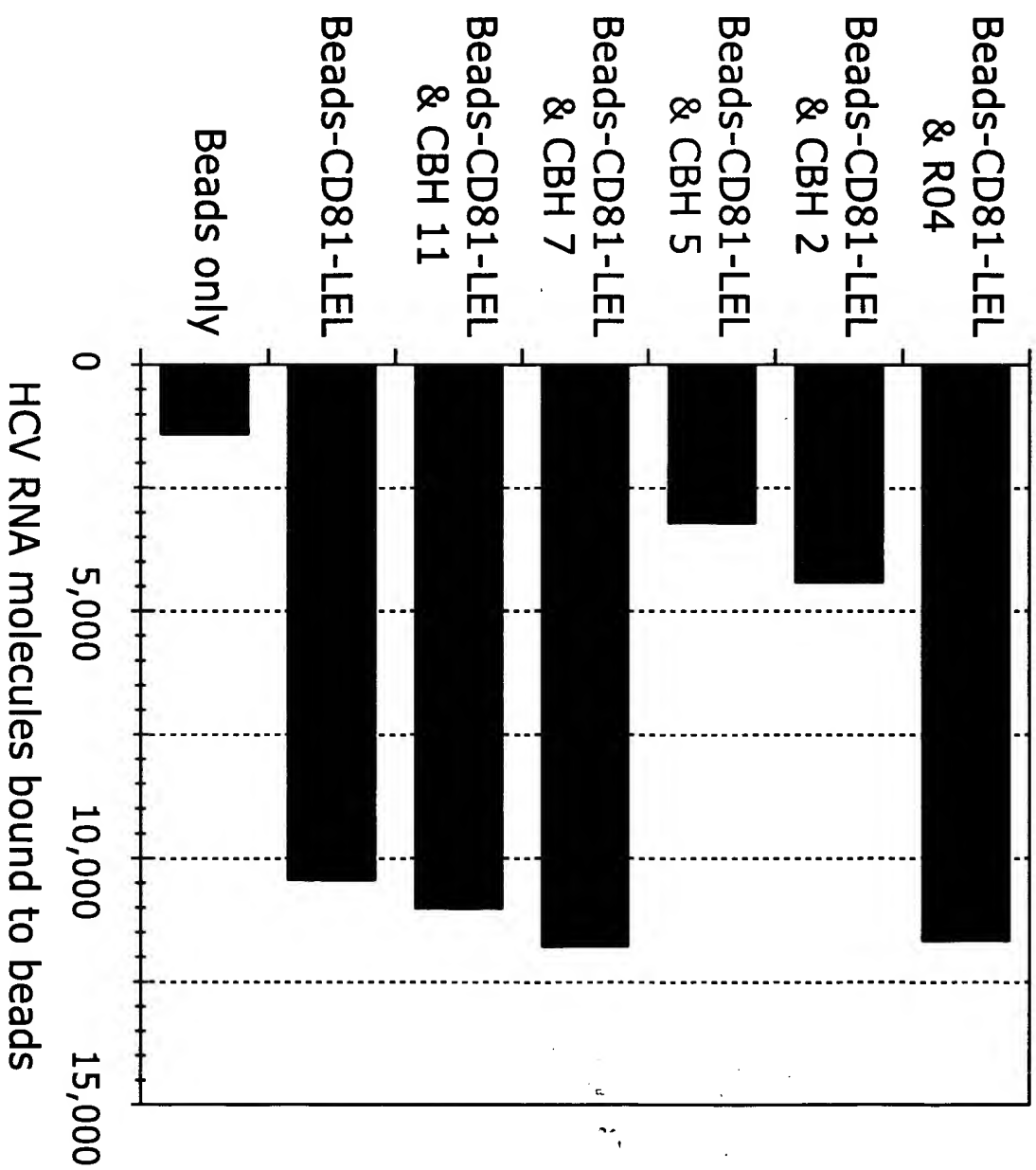


FIG. 17

Inhibition of HCV 1a E2 binding to GNA & CD81

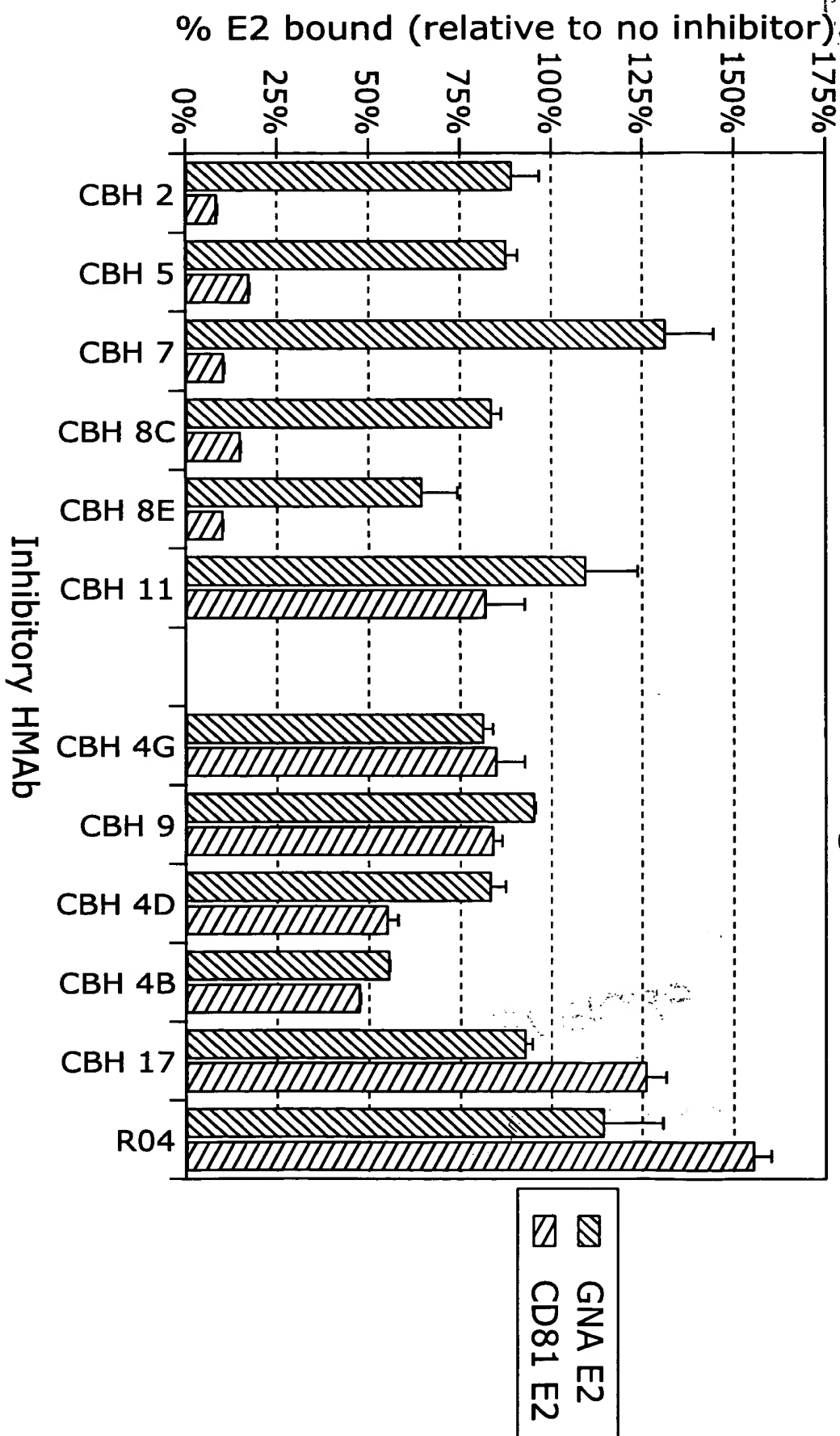


FIG. 18



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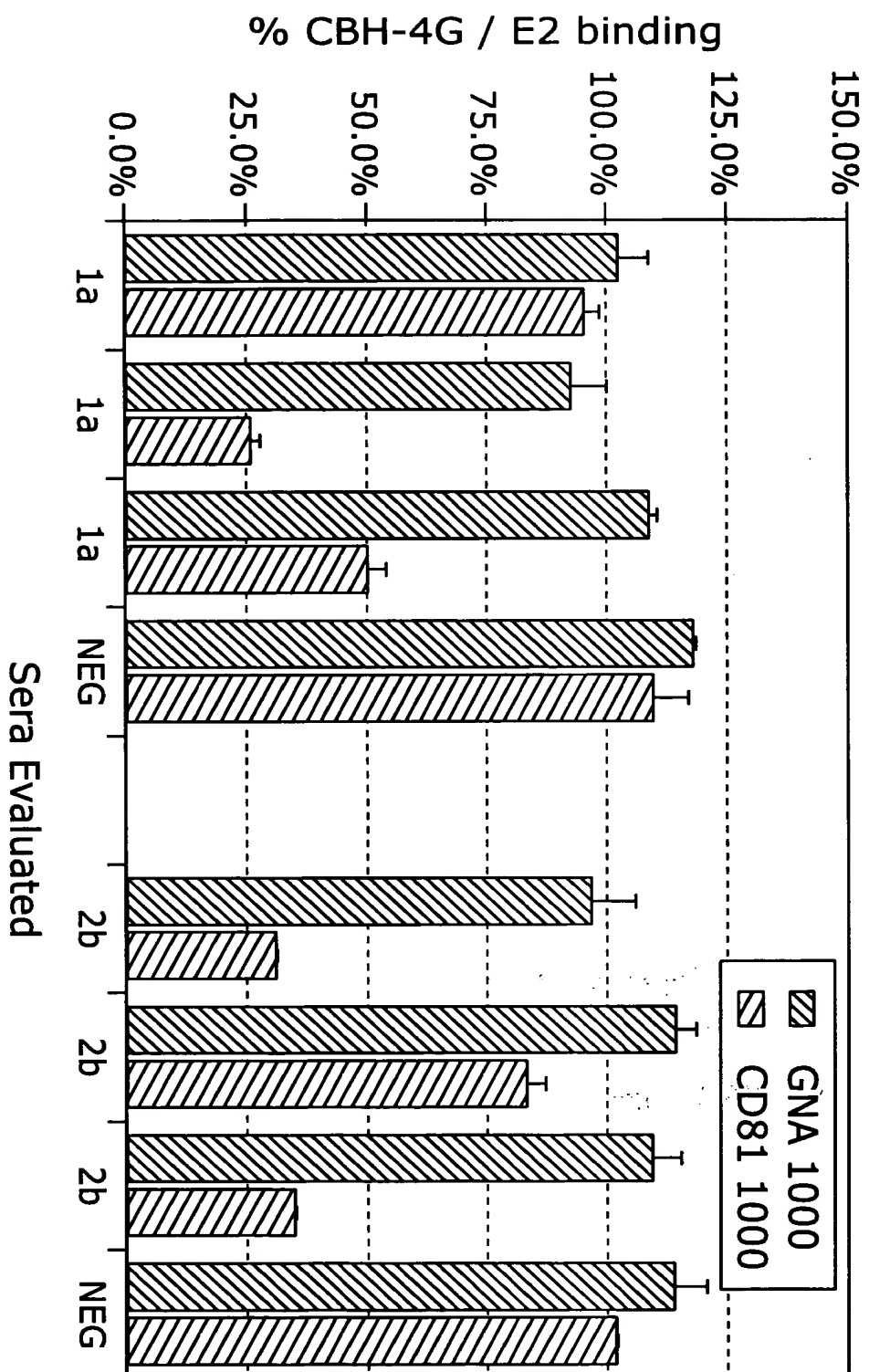
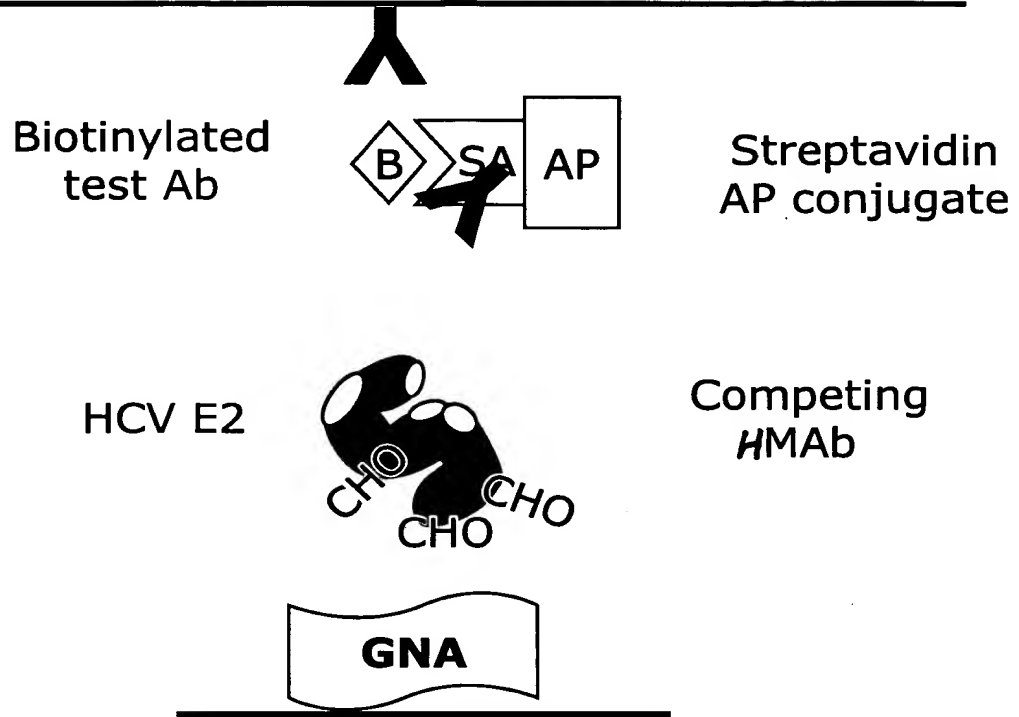


FIG. 19

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Competition Assays



- Coat plates with GNA lectin
- Capture full-length intracellular E2 onto microtiter plate by binding CHO moieties to GNA lectin
- Mix competing HMAb with GNA-captured E2
- Add biotinylated test HMAb. Detect binding of biotinylated test HMAb to E2 with streptavidin-AP conjugate
- Inhibition of binding of test HMAb suggests epitopes within same antibody binding domain

FIG. 20

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Competition Analysis of 4 HCV HMABs

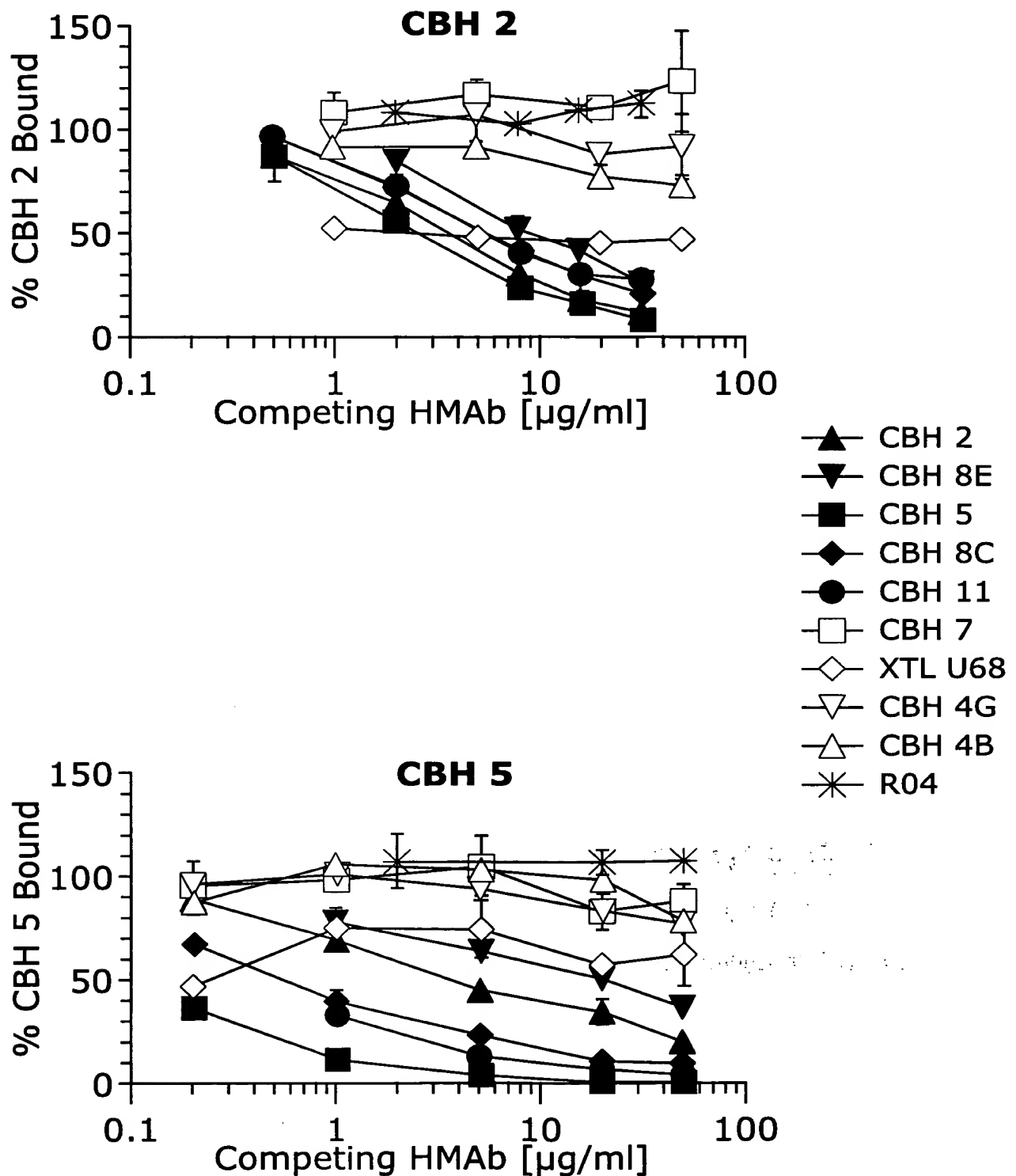


FIG. 21A

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Competition Analysis of 4 HCV HMABs

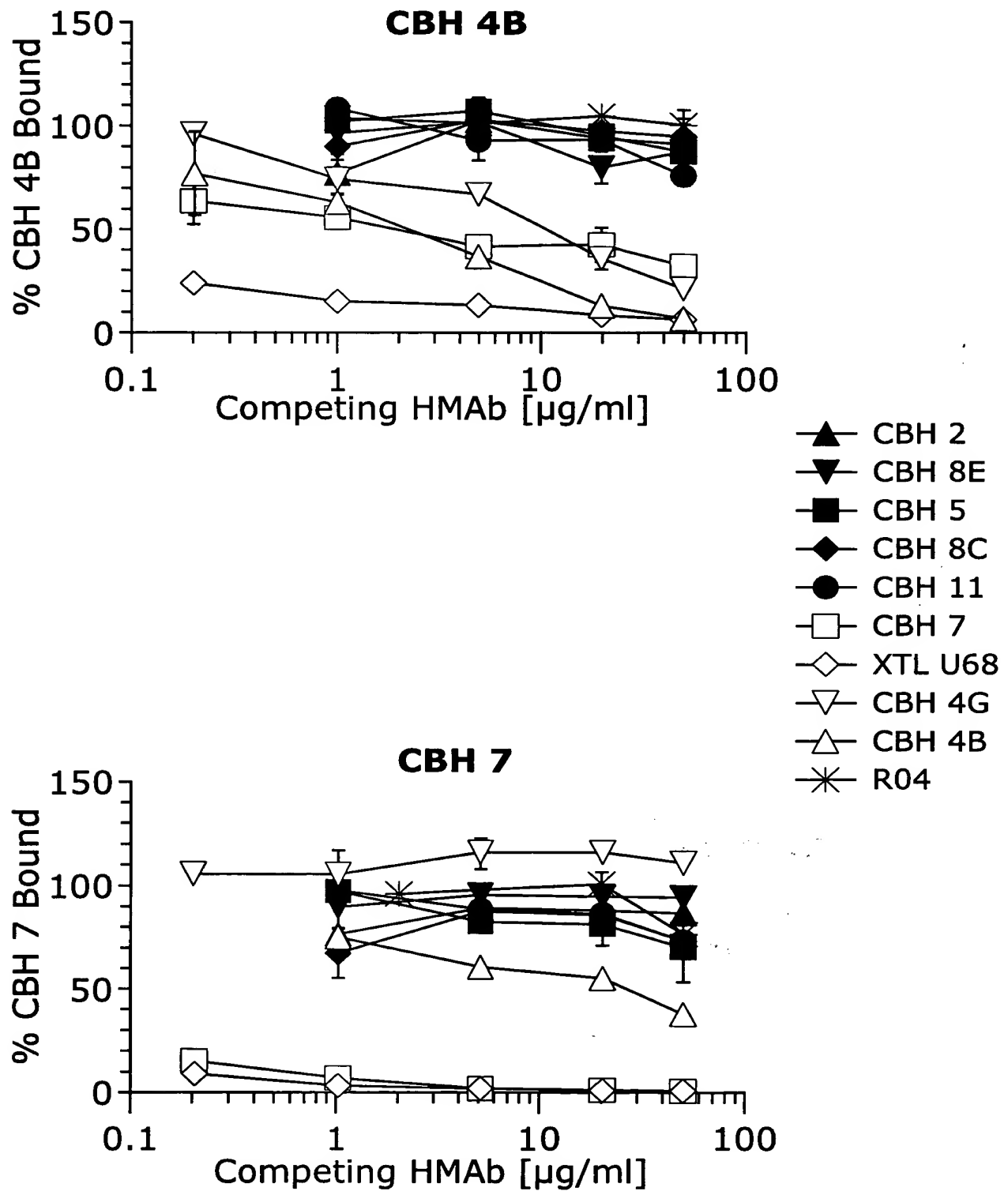


FIG. 21B



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Summary of HMab Competition Analysis

Competitor	Grp	HMab	GROUP I					GRP II	GROUP III	
			CBH 2	CBH 5	CBH 8C	CBH 11	CBH 7	CBH 4G	CBH 4B	
I	CBH 2	1a	18	39	51	ND	93	66	76	
		1b	17	50	50	48	91	84	84	
	CBH 8E	1a	13	39	48	ND	79	63	80	
		1b	23	45	57	51	91	87	78	
	CBH 5	1a	17	9	22	ND	71	60	74	
		1b	4	7	24	9	77	76	80	
	CBH 8C	1a	27	48	25	ND	85	74	84	
		1b	11	23	33	23	84	87	86	
	CBH 11	1a	96	93	84	ND	97	72	87	
		1b	24	25	43	25	82	97	83	
II	CBH 7	1a	40	42	45	ND	2	251	11	
		1b	104	104	89	92	2	146	36	

FIG. 22A

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XTL U68		1a	60	63	108	ND	0	1	2
	1b		39	57	73	66	0	23	9
III	CBH 4G	1a	107	95	85	ND	112	40	68
	1b		87	83	81	87	114	40	44
	CBH 4B	1a	92	92	87	ND	85	24	29
	1b		78	93	66	81	63	34	13
	CBH 4D	1a	98	86	90	ND	135	37	58
	1b		91	82	76	87	102	45	37
IV	CBH 17	1a	94	87	87	ND	114	102	103
	1b		73	101	88	95	92	89	64
C	R04	1a	98	91	92	ND	101	92	98
	1b		96	104	104	101	99	120	101

Scale



60%-140%



Results are the mean percent binding of test antibody relative to wells without any competing antibody. Results are the mean values obtained from 2-5 separate experiments. Both genotype 1a and 1b E2 proteins were tested. ND = not done.

FIG. 22B



HCV E2 Deletion Constructs

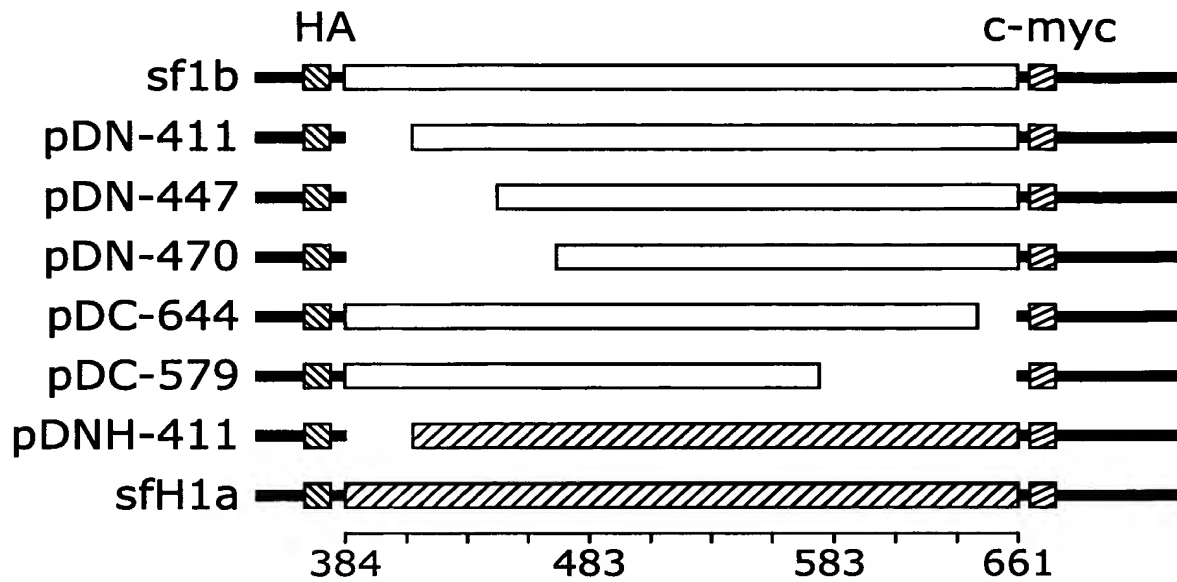


FIG. 23

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HCV E2 Deletion Constructs are efficiently expressed

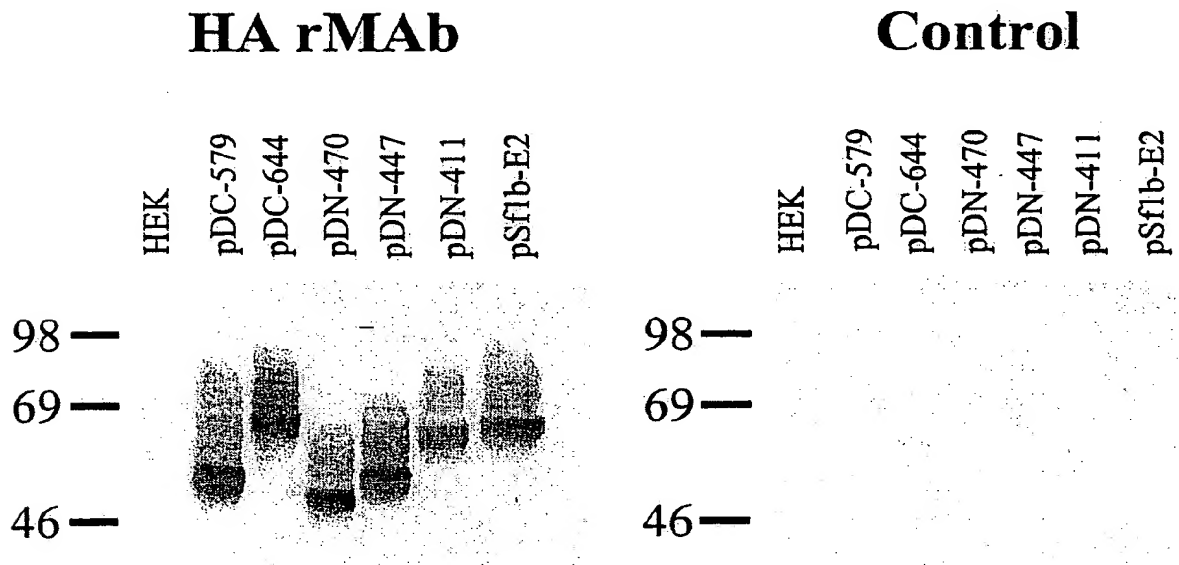
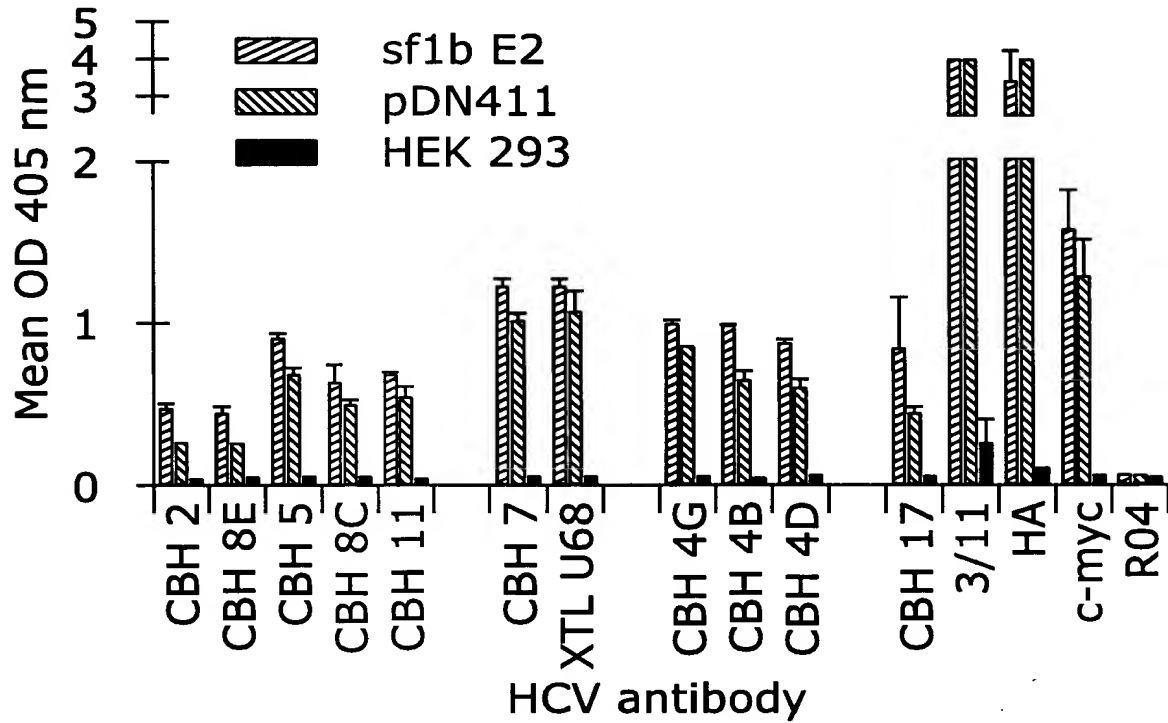


FIG. 24

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HCV E2 Deletion Constructs

HCV 1b E2 w or w/o HVR-1



HCV H1a E2 w or w/o HVR-1

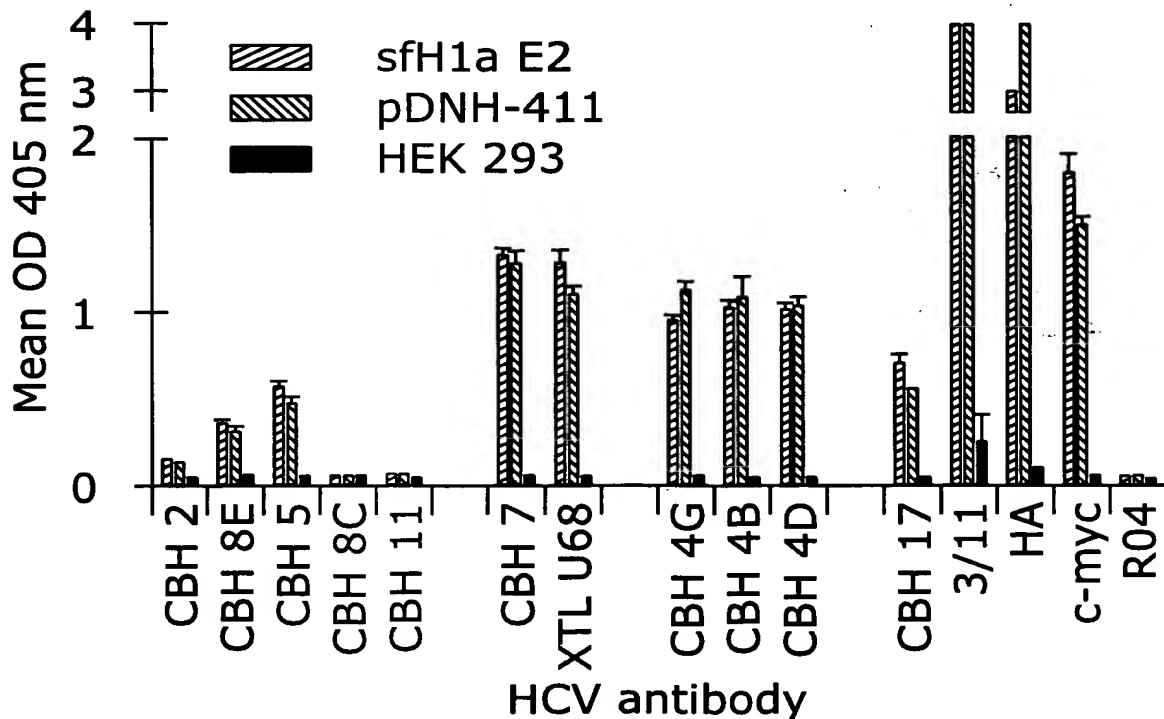


FIG. 25A



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HCV E2 Deletion Constructs

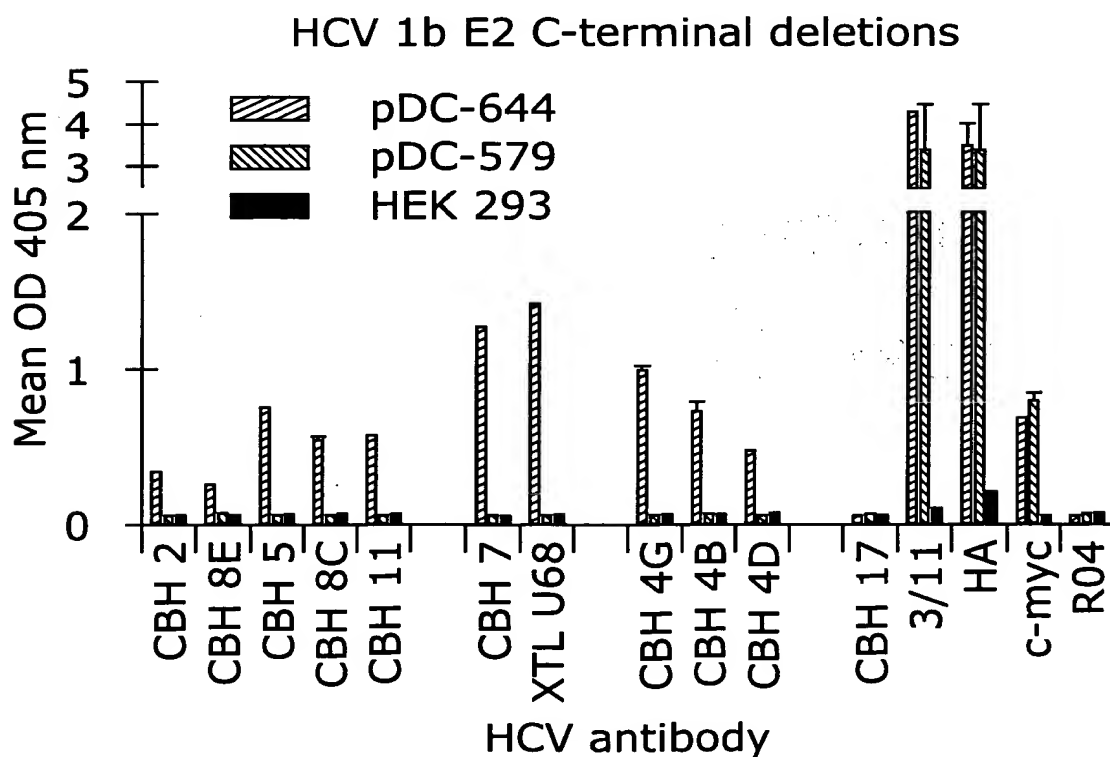
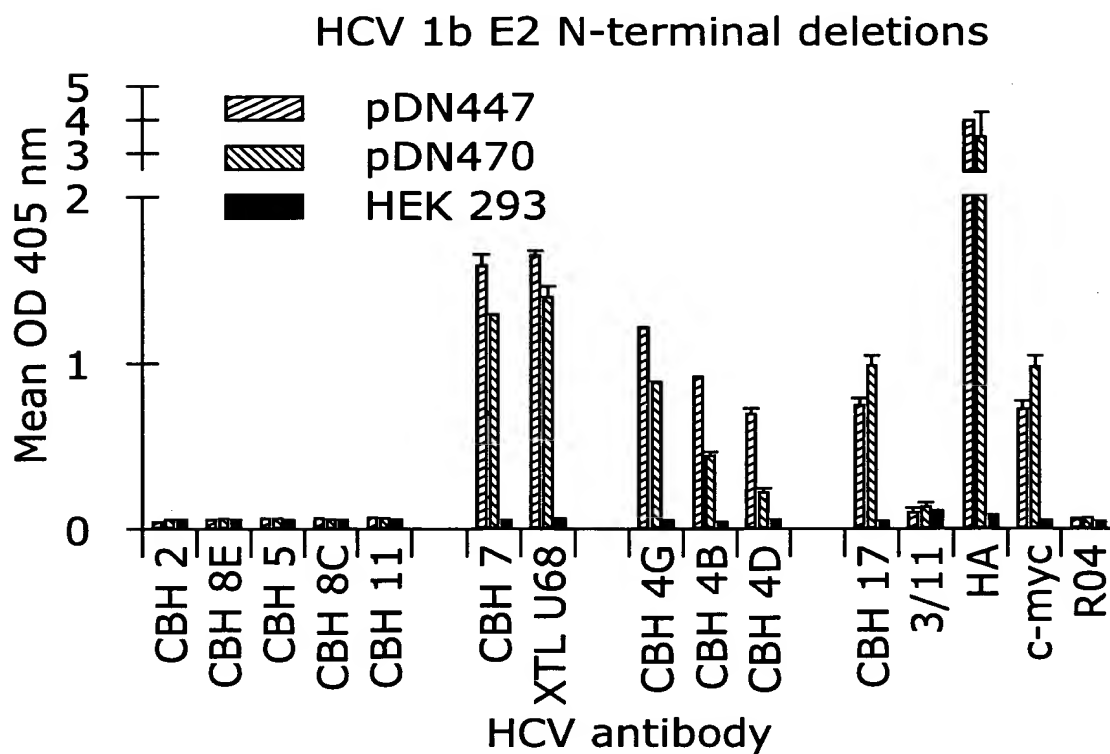


FIG. 25B

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HCV sera have variable levels of antibodies that inhibit CBH-2 & CBH-7

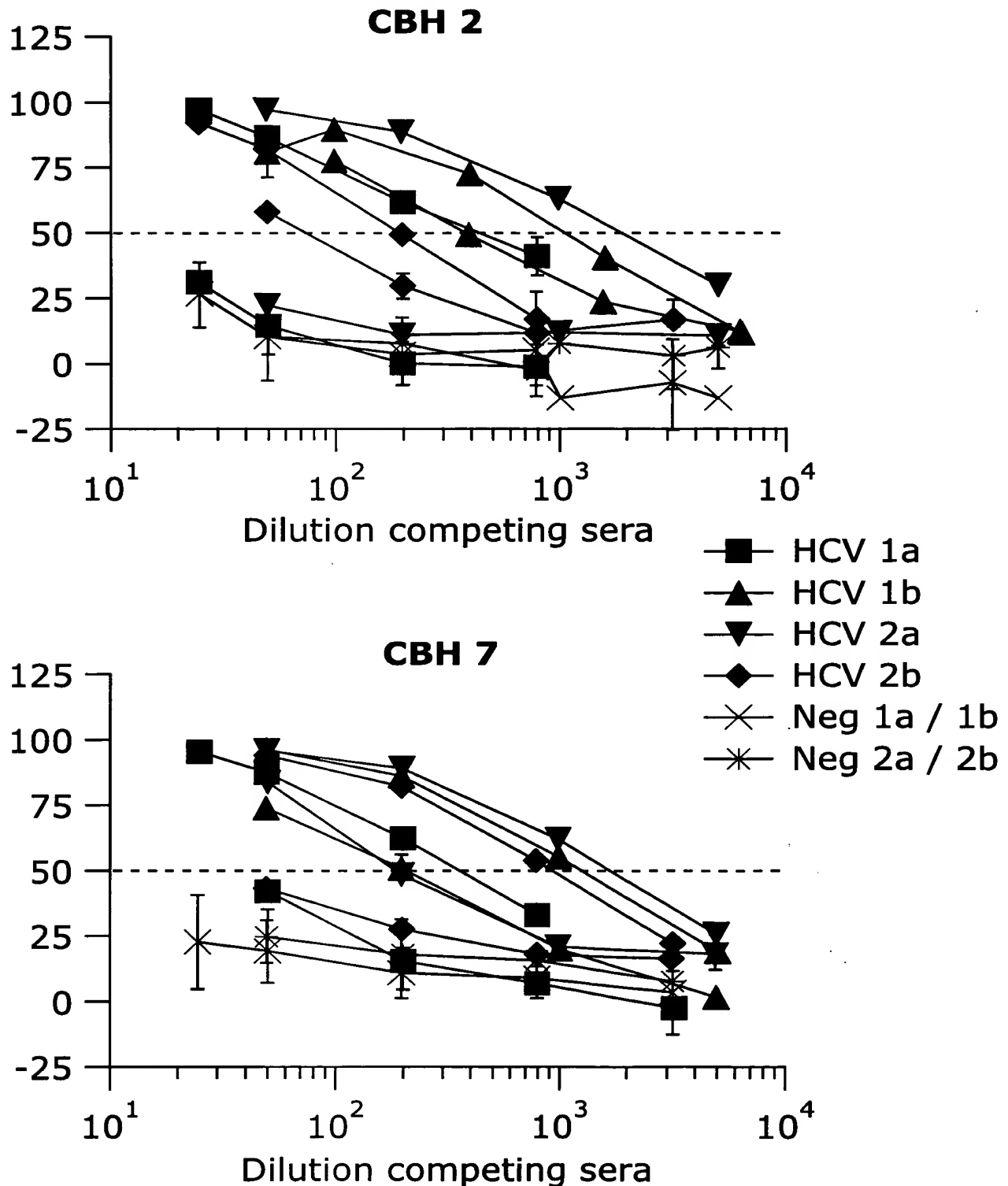


FIG. 26

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HCV sera have variable levels of antibodies
that inhibit CBH-2 & CBH-7

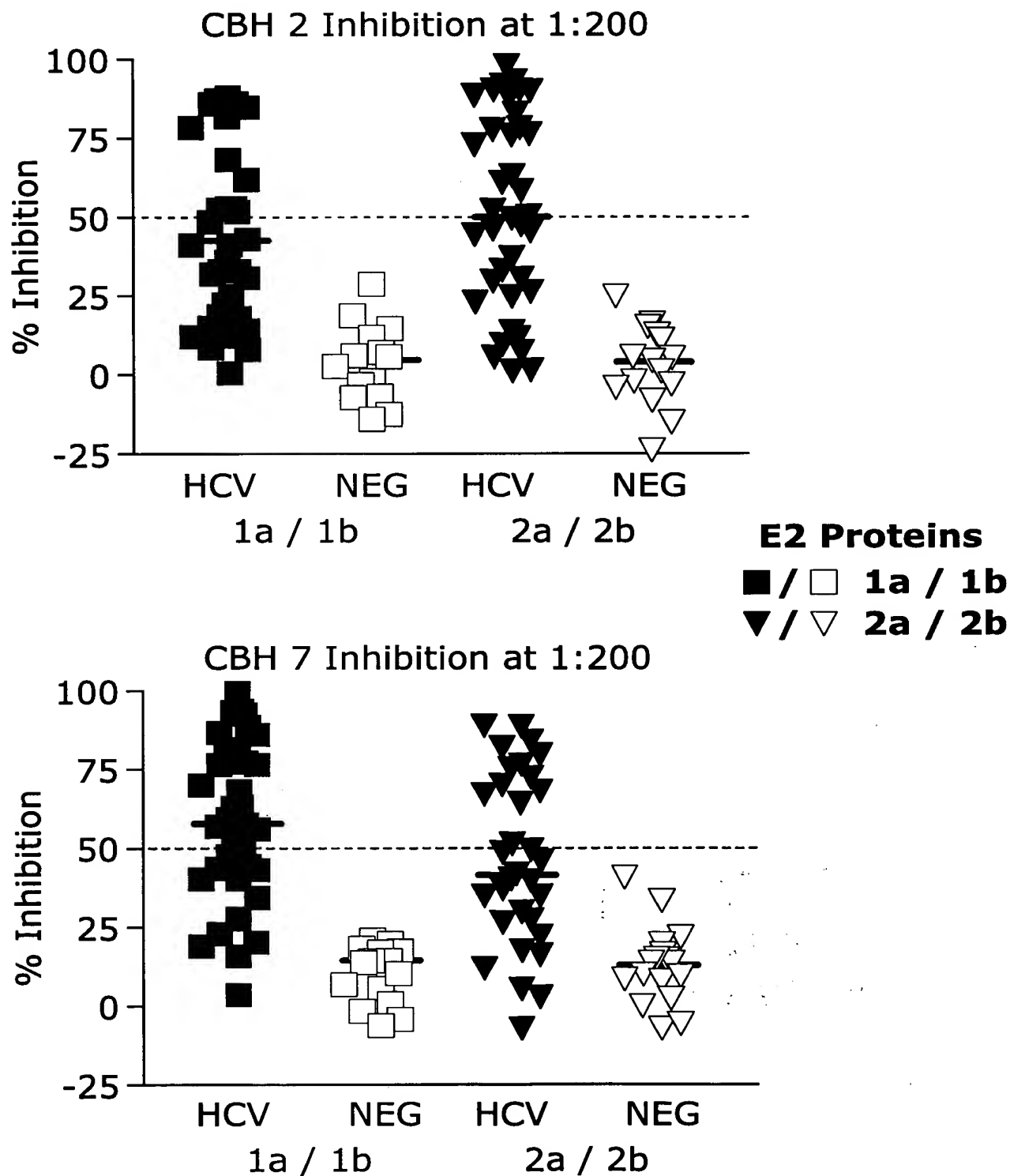
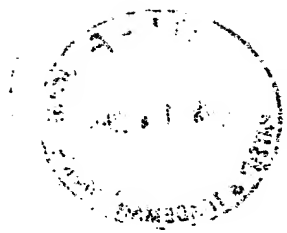


FIG. 27



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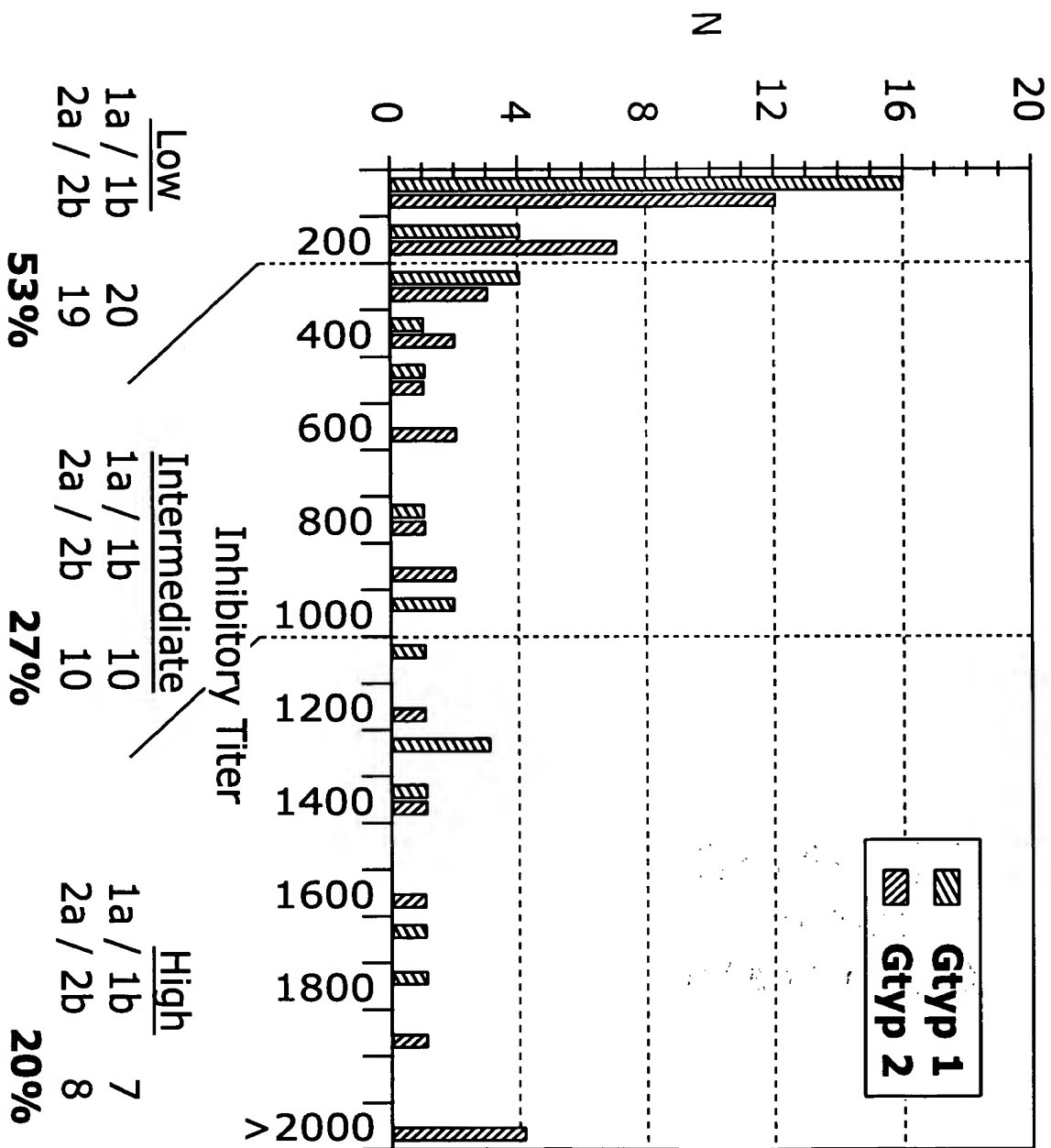


FIG. 28



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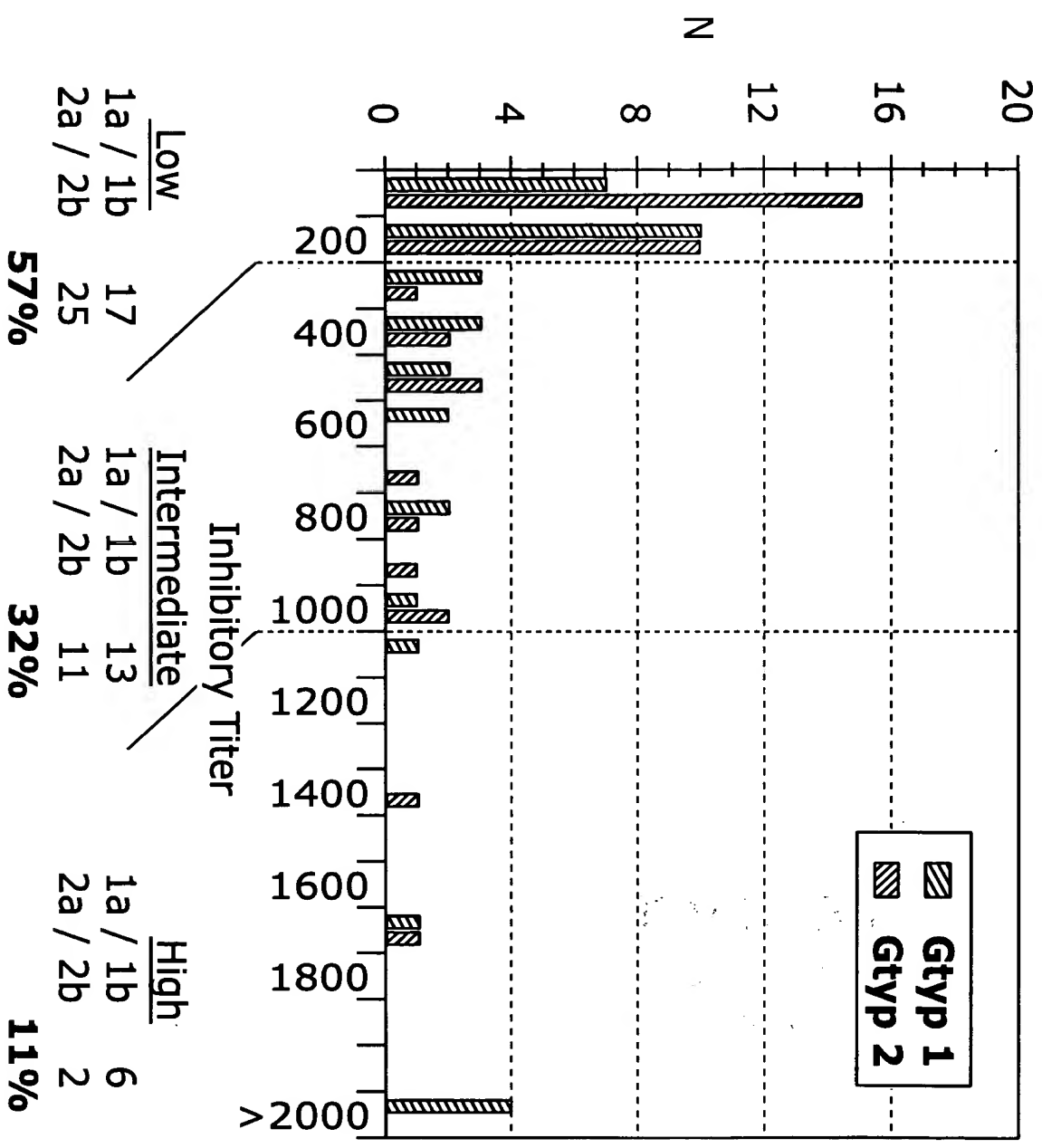


FIG. 29